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(54) Title: USE OF TRICYCLIC ANTIDEPRESSANTS FOR LOCAL ANALGESIA

(57) Abstract

When administered locally, tricyclic, second generation and third generation antidepressants, such as amitriptyline and desipramine, have been shown to produce analgesia in a subject having a site of local discomfort. The analgesic effect of such antidepressants, when administered locally is equal to that achieved by systemic administration and lasts longer. The invention provides compositions containing tricyclic, second generation and third generation antidepressants for local administration, such as those formulated for topical application, or for injection in slow release delivery vehicles, and methods for their use for producing local analgesia.

USE OF TRICYCLIC ANTIDEPRESSANTS FOR LOCAL ANALGESIA

FIELD OF THE INVENTION

5   The present invention relates to therapeutic compositions and methods for
their use. In a particular aspect, the present invention relates to compositions for
producing pain relief and methods of their use.

BACKGROUND OF THE INVENTION

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        Neuropathic pain is a form of chronic pain that can persist for months, years or
decades following an injury, and results from damage to peripheral nerves, nerve
roots, the spinal cord or certain brain regions. It differs from nociceptive pain in
terms of duration, characteristics, underlying mechanisms and treatment (Bennett G.J.

15   (1994a) In: *Textbook of Pain* (Ed. Wall PD, Melzack R) Churchill Livingstone,
London 3rd edn, 201). Neuropathic pain can consist of spontaneous pain (e.g.,
burning, cutting, tingling), evoked pain (e.g., allodynia evoked by stimulation of non-
nociceptive afferents, and hyperalgesia evoked by stimulation of nociceptive
afferents) and paroxysmal pain (e.g., originating from a trigger point, described as

20   stabbing, lancinating, shocklike) (Bennett G.J. (1994a) In: *Textbook of Pain* (Ed. Wall
P.D., Melzack R.) Churchill Livingstone, London 3rd edn, 201). Neuropathic pain
can accompany nociceptive pain, and multiple treatment strategies may be required
for optimal alleviation of pain (Portenoy R.K. (1991) In: *Towards a New
Pharmacology of Pain* (Ed. Basbaum A.I., Besson J.M.) John Wiley & Sons Ltd, New

25   York, 393; Devor M. et al. (1991) In: *Towards a New Pharmacotherapy of Pain* (Ed.
Basbaum A.I., Besson J.M.) John Wiley & Sons, New York, 417). Neuropathic
syndromes are traditionally classified according to the disease or event that
precipitated them (e.g., postherpetic neuralgia following shingles, causalgia following
partial damage to a major nerve, central pain following a thalamic infarct) (Portenoy

30   R.K. (1991) In: *Towards a New Pharmacology of Pain* (Ed. Basbaum A.I., Besson
J.M.) John Wiley & Sons Ltd, New York, 393; Merskey H., Bogaduk N. (1994) In:

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Classification of Chronic Pain. Descriptions of Chronic Pain Syndromes and Definitions of Pain Terms, 2nd edn., IASP Press, Seattle, page 40). The involvement of the sympathetic nervous system in a number of these conditions has been appreciated for some time. Pain syndromes with a sympathetic component are
5   considered as sympathetically maintained pain (reflect sympathetic dystrophy, causalgia) (Bonica J.J. (1990) In: *The Management of Pain* (Ed. Bonica J.J.) Lea & Fibiger, Philadelphia 2nd edn., 220; Blumeberg H., JänigW. (1994) In: *Textbook of Pain* (Ed. Wall P.D., Melzack R.) Churchill Livingstone, London 3rd edn., 685).

10   Neuropathic pain is particularly difficult to treat clinically. The use of opioids is controversial, with issues of contention being the relative refractoriness of neuropathic pain compared to nociceptive pain, the need for higher doses with an increased incidence of side effects to achieve partial effects, and concerns over the long term use of opioids in a non-malignant context (Amér S., Meyerson B.A. (1988)
15   *Pain* 22: 11; Kuypers H., Konig H., Adriaenson H., Gybels J.M. (1991) *Pain* 47: 5; Portenoy R.K., Foley K.M., Inturrisi C.E. (1990) *Pain* 43: 273; Portenoy R.K. (1994) In: *Progress in Pain Research and Management* (Ed. Fields H.L., Liebskind J.C.) IASP Press, Seattle, 247). The major classes of agents currently used to treat neuropathic pain include systemically delivered antidepressants, anticonvulsants, local
20   anesthetics, and specialized agents such as muscle relaxants, and sympatholytic drugs (reviewed Portenoy R.K. (1991) In: *Towards a New Pharmacology of Pain* (Ed. Basbaum A.I., Besson J.M.) John Wiley & Sons Ltd, New York, 393; Portenoy R.K. (1993) *Drug Therapy* 23: 41; Max M.B. (1994) In: *Progress in Pain Research and Management* (Ed. Fields H.L., Liebskind J.C.) IASP Press, Seattle, 229). However,
25   many of these treatments show limited effectiveness (complete pain relief is rarely achieved), and there is a high incidence of debilitative side effects (Portenoy R.K. (1993) *Drug Therapy* 23: 41; Bennett G.J. (1994a) In: *Textbook of Pain* (Ed. Wall P.D., Melzack R.) Churchill Livingstone, London 3rd edn, 201; Mac Farlane et al., (1997*) Pharmacol. Ther.* 75:1).

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Damage to nerves can activate changes in both the peripheral and central nervous systems, and these lead to the characteristic expression of neuropathic pain. Peripherally, the following mechanisms may be involved (reviewed by Devor M et al.

5   (1991) In: *Towards a New Pharmacotherapy of Pain* (Ed. Basbaum A.I., Besson J.M.) John Wiley & Sons, New York, 417; Devor M. (1994) In: *Textbook of Pain* (Ed. Wall P.D., Melzack R.) Churchill Livingstone, London, 3rd edn., 79; Blumeberg H., JänigW.(1994) In: *Textbook of Pain* (Ed. Wall P.D., Melzack R.) Churchill Livingstone, London 3rd edn, 685). (1) Following injury, normally silent nociceptors

10   become sensitized and acquire an ongoing spontaneous activity, a lowered threshold for activation, and a heightened response to suprathreshold stimulation. (2) Development of ectopic discharges can occur at the site of injury, site of regeneration and neuroma formation, or within the dorsal root ganglion. (3) Following injury, sympathetic afferents can excite sensory nerve endings, regenerating nerve sprouts,

15   neuromas, and the dorsal root ganglion cell. Central mechanisms implicated in neuropathic pain are as follows (Coderre et al., (1993) *Pain* 52:259; Bennett (1994) In: *Textbook of Pain* (ed. by P.D. Wall et al., London, 3rd edn., 201; Woulff and Doubell (1994) *Curr. Opin. Neurobiol.* 4:525): (1) Transmission neurons within the spinal cord exhibit enhanced sensitivity to excitation by excitatory amino acids

20   (EAAs) and substance P (SP). This condition leads to allodynia (following Aβ fibre activation) and hyperalgesia (following Aδ and C fibre activation). (2) Disinhibition resulting from loss of inhibitory input to projection neurons and an aberrant patterning of responses to large diameter afferent activation. (3) Myelinated afferents, which normally would enervate deeper laminae, projecting into and making synaptic

25   connections in more superficial laminae. This can be a mechanism for the generation of allodynia.

A number of models of neuropathic pain have been developed in order to provide a basis for understanding of neuropathic changes that occur following nerve

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injury, and to provide model systems in which to test novel therapeutic strategies (reviewed Bennett G.J. (1994b) In: *Progress in Pain Research and Management* Vol 2 (Ed. Gebhart C.F., Hammond D.L, Jensen T.S.) IASP Press, Seattle, 495). The best characterized are a number of nerve injury models which have been developed

5   only relatively recently (chronic constriction injury due to loose ligation of the sciatic nerve, Bennett G.J., Xie Y.K. (1988) *Pain* 33: 87; and partial sciatic ligation due to tight ligation of a portion of the sciatic nerve (Seltzer et al. (1990) *Pain* 43:205) and tight ligation of two spinal nerves (Kim S.H., Chung J.M. (1992) *Pain* 50: 355)). These model systems exhibit various manifestations of neuropathic pain (e.g., degree

10   of expression of spontaneous pain behaviors, mechanical or thermal allodynia, mechanical or thermal hyperalgesia) and degree of sympathetic nerve involvement (Neil A., et al. (1991) *Brain Res* 565: 237; Shir Y., Seltzer Z. (1991) *Pain* 45: 309; Kim S.H., Na H.S., Sheen Ki, Chung J.M. (1993) *Pain* 55: 85; Kim K.J., Yoon Y.W., Chung J.M. (1997) *Exp Brain Res* 113: 200). The spinal nerve ligation (SNL) model

15   exhibits a greater degree of evoked pain (mechanical allodynia) and of sympathetic involvement in this parameter than do the partial sciatic ligation or chronic constriction injury models, while the chronic constriction injury model exhibits a greater degree of spontaneous pain. It is however appreciated that such properties may be relative and change with time (i.e., sympathetic dependency can change with

20   time both in animal models and in clinical neuropathic pain, Staton-Hicks M, et al. (1995) *Pain* 63: 127).

Systemically administered antidepressants offer an alternate therapy in neuropathic and chronic pain states. Interactions with biogenic amines, endogenous

25   opioids, excitatory amino acid receptors, substance P and calcium and sodium channels have been considered in efforts to pinpoint the mechanism of systemically administered antidepressants (reviewed by Eschalier A. et al. (1994) *CNS Drugs* 2: 261). What is clear is that antidepressants can act at both supraspinal (Spiegel, K. et al., *Ann. Neurol.* 13 (1983) 462-465, Eschalier A, et al. (1994) *CNS Drugs* 2: 261)

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and spinal (Hwang, A.S. and Wilcox, G.L., *Pain* 28 (1987) 343-355; Iwashita, T.
and Shimizu, T., *Brain Research* 581 (1992) 59-66; Eisenachand J. and Gebhart
G.F., *Anesthesiology* 83 (1995) 1046-1054) sites of action.

5   This analgesic action is independent of antidepressant effects as it occurs in
non-depressed subjects and occurs independently of mood changes in depressed
subjects (Magni G. (1991) *Drugs* 42: 730; Onghena P. and Van Houdenhove B (1992)
Pain 49: 205; Max M.B. (1994) In: *Progress in Pain Research and Management* (Ed.
Fields H.L., Liebskind J.C.) IASP Press, Seattle, 229, McQuay H.J., Tramer M., Nye

10   B.A., Carroll D., Wiffen P.J., Moore R.A. (1996) *Pain* 68: 217). Agents which block
the uptake of both noradrenaline (NA) and 5-hydroxytryptamine (5-HT)
such as amitriptyline, or which block NA but not 5-HT, such as desipramine, are
more effective than those with selectivity for 5-HT, such as fluoxetine (Max M.B.
(1994) In: *Progress in Pain Research and Management* (Ed. Fields H.L., Liebskind

15   J.C.) IASP Press, Seattle, 229). Pain relief is reported to be apparent within one week
of therapy (McQuay H.J., Carroll D., Glynn C.J. (1992) *Anaesthesia* 47: 646). This
time course corresponds to the time required to attain stable plasma levels (t½ 17-36
hours in humans, Ziegler V.E., Biggs J.T., Aardekani A.B., Rosen S.H. (1978) *J Clin
Pharmacol* 18: 462). By contrast. the antidepressant activity of these compounds

20   takes 4-6 weeks to become apparent (Potter W.Z., Rudorfer M., Manji H. (1991) *New
Eng J Med* 325: 633). These differences in profile of active drugs, time course, and
independent expression of effects suggest that mechanisms underlying pain relief and
alleviation of depression differ.

25   In animal tests, both the systemic and spinal administration of antidepressants
show intrinsic efficacy in a number of nociceptive pain tests, and augment analgesia
produced by opioids (reviewed by Eschalier A, Mestre C, Dubray C, Ardid D (1994)
CNS Drugs 2: 261). However, this profile can be variable, and inhibitory effects on
the action of morphine have been observed in some cases (reviewed by Eschalier et

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al., supra). Methodological issues (e.g., test paradigm, intensity of stimulus, dose, regimen of acute versus chronic administration) are reported to account for many of these differences (Kellstein D.E., Malseed R.T., Goldstein F.J. (1984) *Pain* 60: 275; Kellstein D.E., Malseed R.T., Ossipov M.H., Goldstein F.J. (1988)

5   *Neuropharmacology* 27: 1; Fialip J., MartyH., Makambila M.C., CiViate M.A., Eschalier A.(1989) *J Pharmacol Exp Ther* 248: 747). Systemically administered antidepressants also exhibit intrinsic actions in a number of neuropathic pain tests including nerve transaction (Seltzer Z., Tal M., Sherav Y. (1989) *Pain* 37: 245), mononeuropathy (Ardid D, Gilbaud G (1992) *Pain* 49: 279) and diabetic neuropathy

10   models (C.Courteix et al.(1994) *Pain* 57:153-160). One study examined chronic versus acute dosing regimens (Ardid D., Gilbaud G. (1992) *Pain* 49: 279), and observed that the activity seen following chronic paradigms appeared to be accounted for by accumulating doses rather than being qualitatively different.

15   SUMMARY OF THE INVENTION

In accordance with the present invention, it has been discovered that locally administered tricyclic, second generation, or third generation antidepressant(s) produce a local antinociceptive action, especially against inflammatory and

20   neuropathic pain. When administered locally in animal models of inflammatory (formalin test) and neuropathic pain (spinal nerve ligation), amitriptyline, a non selective noradrenaline (NA) and 5-hydroxytryptamine (5-HT) reuptake inhibitor, and desipramine, a selective NA reuptake inhibitor, produced local antinociceptive actions.

25

Accordingly, in one aspect of the invention, there are provided compositions containing an effective therapeutic amount of a tricyclic, second generation or third generation antidepressant in an inert carrier. The compositions are preferably formulated for local administration, for example in a saline solution, or as a cream,

30   gel, ointment, or spray.

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Such formulations possess the advantage that a higher and more efficacious concentration of the antidepressant can be attained in the region of the sensory nerve terminal than with systemic administration of the antidepressant. In addition, local
5   administration greatly reduces the side effects that result from systemic administration of tricyclic, second generation or third generation antidepressant(s).

In another aspect of the invention, there are provided methods for producing local analgesia in a subject having a site of local discomfort, such as is caused by
10   inflammatory or neuropathic conditions. In the invention method, local analgesia is obtained by locally administering an effective amount of a tricyclic, second generation or third generation antidepressant to the site of discomfort, for example by injection, supersonic powder injection, transdermal electroporation, topical application, or encapsulation in a slow release delivery vehicle.
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<u>BRIEF DESCRIPTION OF THE FIGURES</u>

Figure 1 is a graph illustrating the difference in the threshold pain response over 22 days in the ipsilateral and contralateral paws of rats subjected to nerve
20   ligation. The row of bars at the back of the figure illustrates the static mechanical allodynia 50% withdrawal threshold (grams) of nerve-ligated rats, and the row of bars at the front of the figure illustrates the thermal hyperalgesia (seconds) on successive days following spinal nerve ligation. Values depict group means (n=6 per group).

25   **Figures 2A-C** are a series of graphs illustrating the time course of the effect of systemic amitriptyline (AMI) at various doses on thermal hyperalgesia in the ipsilateral paw of nerve-ligated rats (Figure 2A), and the thermal withdrawal threshold in the contralateral paw (Figure 2B), with ●=saline control; ■=1mg/kg AMIT; ▲=3 mg/kg AMI, and ▼=10 mg/kg AMI. Figure 2C illustrates the cumulative change in

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the Maximum Possible Effect (ΔMPE) time course for ipsilateral (ipsi) and
contralateral (contra) paws, with open bars representing saline control, bars with
rightward sloping lines representing 1 mg/kg AMIT, bars with leftward sloping lines
representing 3 mg/kg AMI, and bars with crosshatching representing 10 mg/kg AMI.

5   Values represent the MPE ∓s.e.m. (n=5 for 1 and 3 mg/kg, and n=15 for 10 mg/kg).
*=P<.05 compared to saline controls.

Figures 3A-C are a series of graphs illustrating the time course of the effect of
spinal amitriptyline (60 μg) on thermal hyperalgesia in the ipsilateral paw (Figure

10   3A), and the thermal withdrawal threshold in the contralateral paw (Figure 3B), with
●=saline control, ■= 60μg AMIT. Figure 3C represents the cumulative change in the
MPE (ΔMPE) time course in ipsilateral (ipsi) and contralateral (contra) paws, with
open bars representing saline control, rightward sloping bars representing 100 nmol
AMI (ipsi), and leftward sloping bars representing 100 nmol AMI (contra). Values

15   represent the MPE ∓ s.e.m. (n=6 for both groups). *=P<.05 compared to saline
controls.

Figures 4A-C are a series of graphs illustrating the time course of the effect of
local injection of 100 nmol amitriptyline on thermal hyperalgesia. Figure 4A shows

20   the effect in the ipsilateral paw when the antidepressant is injected into the nerve-
injured paw (ipsi) and non-injured paw (contra), and Figure 4B shows the time course
of the change in the thermal withdrawal latency in the contralateral paw, when the
antidepressant is injected into the nerve injured paw (ipsi) and non-injured paw
(contra), with ●=saline control; ■=AMI ipsi, and ; ▲= AMI contra. Figure 4C

25   depicts the cumulative change in MPE value (ΔMPE), with open bars representing
saline control (ipsi), bars with rightward sloping lines representing 100 nmol AMI
(ipsi), and bars with leftward sloping lines representing 100 nmol AMI (contra).
Values represent the MPE ∓ s.e.m. (n=9 for all groups). *=P<.05 as compared to
saline controls, *t* =P<.01 as compared to contralateral controls.

Figures 5A-C are a series of graphs illustrating the time course of the effect of systemic amitriptyline on static mechanical allodynia in the ipsilateral paw (Figure 5A) and on the threshold of withdrawal to static mechanical stimuli in the

5   contralateral paw (Figure 5B), with ●=saline control; ■=1.5 mg/kg AMI; ▲=5 mg/kg AMI, and ▼=10 mg/kg AMI. Figure 5C represents the cumulative change in the MPE (ΔMPE), with open bars representing saline control, bars with rightward sloping lines representing 1.5 mg/kg AMI, bars with leftward sloping lines representing 5 mg/kg AMI, and bars with crosshatching representing 10 mg/kg AMI. Values

10   represent MPE ∓ s.e.m. (n=5 for 1.5 and 5 mg/kg, and n=15 for 10 mg/kg). *=P<.05 as compared to saline, *t* =P<.05 as compared 1.5 and 5.0 mg/kg.

Figures 6A-C are a series of graphs illustrating the time course of the effect of spinal amitriptyline on static mechanical allodynia in the ipsilateral paw (Figure 6A),

15   on the threshold of withdrawal to static mechanical stimuli in the contralateral paw (Figure 6B), with ●=saline control; ■=30μg AMI, ▲=60 μg AMI , and ▼=90 μg AMI. Figure 6C illustrates the cumulative change in the MPE (ΔMPE),), with open bars representing saline control; bars with rightward sloping lines representing 30μg AMI, bars with leftward sloping lines representing 60 μg AMI, and bars with

20   crosshatching representing 90 μg AMI. Values represent the MPE ∓ s.e.m. (n=3 for 30 μg and 90 μg , and n=5 for 60 μg). *=P<.05 compared to saline controls.

Figures 7A-C are a series of graphs illustrating the time course of the effect of local peripheral injection of amitriptyline (100nmol) on mechanical allodynia. Figure

25   7A illustrates the effect in the ipsilateral paw when the antidepressant is injected locally into the nerve-injured paw (ipsi) and non injured paw (contra). Figure 7B illustrates the time course of the change in the mechanical threshold of the contralateral paw following injection into the nerve-injured paw (ipsi) and the non-injured paw (contra), with ●=saline control; ■=AMI (ipsi), and ; ▲= AMI (contra).

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Figure 7C illustrates the cumulative change in the value of MPE (ΔMPE) over the

entire time course), with open bars representing saline control (ipsi), bars with

rightward sloping lines representing 100 nmol AMI (ipsi), and bars with leftward

sloping lines representing 100 nmol AMI (contra). Values represent the MPE ∓ s.e.m.

5   (n=9 for all groups). *=P<.05 as compared to saline controls, and t=P<.01 as

compared to contralateral controls.

Figures 8A-B are two graphs illustrating the time course of the local

antinociceptive effect produced by coadministration to rats of amitriptyline (AMI)

10   with formalin 2.5% as measured by number of flinches (Figure 8A) or time spent

biting or licking (Figure 8B). ●=2.5% formalin alone; open triangle = 2.5%

formalin+10nM AMI; open square=2.5% formalin+30 nmol AMI; open

diamond = 2.5% formalin+100 nmol AMI. Values depict group means (n=5-11 per

group); error bars for s.e.m. were omitted in the interest of clarity.

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Figures 9A-C are a series of bar graphs illustrating the cumulative scores for

phase 1 flinches (Figure 2A), phase 2 flinches (Figure 9B), and biting/licking time

(Figure 9C) following coadministration to rats of amitriptyline (100 nmol) with 2.5%

formalin (F). The antinociceptive effect of amitriptyline administered to the

20   ipsilateral paw (local effect) was not reproduced by administration of amitriptyline

into the contralateral (CONT) hindpaw. The values depicted are ∓ s.e.m. values (n=5-

11). *=P<0.05, **=P<0.01, and ***=P<0.001 compared to the formalin alone group.

Figures 10A-C are a series of graphs illustrating the inhibitory effect on

25   phase 1 flinches (Figure 10A), phase 2 flinches (Figure 10B), and time spent biting

and licking (Figure 10C) following coadministration to rats of amitriptyline

(100 nmol) (open diamonds) on formalin-induced behaviors at different

concentrations of formalin (filled circles). n=6-11 per group. *=P<0.05, **=P<0.01,

and ***=P<0.001 compared to the formalin only group.

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Figures 11A-C are a series of graphs illustrating partial reversal of the local
antinociceptive action of amitriptyline in the 2.5% formalin (F) test by
coadministration with caffeine (CAFF) as measured by phase 1 flinches (Figure 11A),
5   phase 2 flinches (Figure 11B), and time spent licking and biting (Figure 11C). n=10-
15 except for the caffeine (1500 nmol) group where n=5. *=P<0.05, ***=P<0.001
compared to the formalin only group; t=P<0.05, tt=P<0.01 compared to
formalin/amitriptyline group. The filled bars illustrate 2.5% formalin alone, the
empty bars illustrate administration of AMI alone, the bars with leftward sloping lines
10   illustrate AMI+500 nmol CAFF, and the crosshatched bars illustrate 1500 nmol
caffeine alone or with 100 nmol AMI.

Figures 12A-B are two graphs illustrating the time course of caffeine (CAFF)
reversal of amitriptyline (AMI) action in the 2.5% formalin (F) test for the experiment
15   illustrated in Figures 4A-C as measured by number of flinches (Figure 12A) and time
spent biting and licking (Figure 12B). ●=2.5% formalin alone; open upward-pointing
triangle=2.5% F+100 nmol AMIT; ■=2.5% F+100 nmol AMI+1500 nmol CAFF;
♦=2.5% F+1500 nmol CAFF. Values depict group means (n=5-11 per group); error
bars for s.e.m. were omitted in the interest of clarity.
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Figures 13A-B are graphs illustrating the reversal of the antinociceptive action
of amitriptyline (AMI) against 1.5% formalin (F) by caffeine (CAFF) (Figure 13A),
and 8-cyclopentyl-1,3-dimethylxanthine (CPT) (Figure 13B). n=5-6 per group.
**=P<0.01, ***=P<0.001 compared to formalin alone; t=P<0.05, ttt=P<0.001
25   compared to formalin/amitriptyline. The filled bars represent the effect of
administering 1.5% formalin alone; the bars with downward sloping lines represent
CAFF alone or in combination with AMIT, and the open bars represent the effect of
AMI alone.

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Figure 14 is a bar graph illustrating enhancement of the local antinociceptive
of 30 nM and 100 nM amitriptyline (open bars) against 2.5% formalin (filled bar)
action as measured by decrease in phase 2 flinches in the formalin test by
coadministration of the adenosine kinase inhibitor NH_2dAD (100nmol) (bars with
rightward sloping lines), but not by the adenosine deaminase inhibitor
2'-deoxycoformycin (DCF) (100 nmol) (bars with cross hatching). n=6-8 per group.
*=$P<0.05$ compared to formalin alone; t=$P<0.05$ compared to the
formalin/amitriptyline combination.

Figures 15A-B are bar graphs illustrating inhibition of responses by rats to
2.5% formalin (filled bars) when the formalin is coadministered with MK-801 (20 nM
or 100 nM) (open bars) into the ipsilateral (I) paw, or the contralateral (C) paw. n=6-8
per group.
*=$P<0.05$; **=$P<0.01$, and ***=$P<0.001$ compared to formalin alone.

Figure 16A-B are bar graphs illustrating that the antinociceptive action of
amitriptyline (20nmol and 100nmol) against 2.5% formalin (F) (dark bar) was not
decreased by coadministration of phentolamine (PTA) (100nmol and 500nmol) (bars
with crossed hatching) or naloxone (NLX) (100nmol and 300 nmol) (bars with
horizontal lines). n=5-8 per group.
*=$P<0.05$, ***=$P<0.001$ compared to the formalin group; t=$P<0.05$ compared to the
formalin/amitriptyline combination.

Figures 17A-C are a series of graphs showing dose-related inhibition of
formalin-induced pain response behaviors by locally administered tricyclic
antidepressants. The bar graphs depict the control values for formalin (solid bar) and
saline (open bar) when injected contralaterally; while the dosage graphs depict the
results when antidepressant, rather than control, is injected. Figures 17A and 17B
show amelioration of phase 1 and phase 2 flinching behaviors, respectively, and

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Figure 17C shows amelioration of biting/licking behaviors induced by formalin 2.5%
(■) injected into the dorsal hindpaw of the rat when the formalin is coadministered
with desipramine (DES) or fluoxetine (FLUOX) into the dorsal hindpaw (local effect)
or injected into the contralateral hindpaw (systemic effect). ▲=ipsilateral DES; open

5   triangle=contralateral DES; ●=ipsilateral FLUOX; O=contralateral FLUOX. Data
depicts mean ∓ s.e.m. for n=5-6; n=8 for ipsilateral control). *=P<0.05, **=<0.01,
***=P<0.001 compared to corresponding formalin group.

        Figures 18A-D are a series of graphs illustrating the suppression of pain

10   related behaviors by coadministration of desipramine with 2.5% formalin.
Figures 18A and 18B show the time course for the suppression of flinching behaviors
by coadministration of DES with the formalin into the ipsilateral (Figure 18A) or
contralateral (Figure 18B) paw. Figures 18C and 18D show the time course for
suppression of biting/licking, behaviors after administration of the highest doses

15   injected into the ipsilateral (Figure 18C) or contralateral (Figure 18D) paw. Values
are means, with error bars omitted in the interest of clarity. Data corresponds to
Figure 17.

        Figures 19A-C are a series of graphs illustrating the results of local injection

20   of desipramine (DES) (100 nmol) and fluoxetine (FLX) (100 nmol). Figure 19A
depicts the local antinociceptive action of desipramine, but not fluoxetine, when
injected into the ipsilateral (ipsi) paw corresponding to the spinal nerve ligation, and
Figure 19B depicts the lack of effect in the contralateral paw (contra) with ●=saline
control; ■=DES; and ▲=FLUOX. Figure 19C shows the cumulative change in MPE

25   with the open bars representing saline control, the rightward sloping bars representing
DES and the leftward sloping bars representing FLUOX. n= 9 per group. *=P<0.05
compared to saline group.

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Figures 20A-B are two graphs illustrating the effect of caffeine (1500 nmol) (CAFF) for inhibiting the antinociceptive effect of desipramine when coadministered into the paw ipsilateral to spinal ligation. Figure 20A shows the anti-hyperalgesia MPE over time wherein ●=100nmol DES; ■= 1500 nmol CAFF; and ▲= DES +

5   CAFF. Figure 20B shows the cumulative change in MPE (Δ MPE) wherein the open bar represents 100nmol DES; the bar with rightward sloping lines represents 1500 nmol CAFF; and the bar with crosshatching represents DES + CAFF. n=9 per group; *=P<0.05 compared to saline group.

10   Figures 21A-C are a series of graphs showing a lack of suppression of the local antinociceptive effect of desipramine (DES) or fluoxetine (FLUOX) caused by coadministration of caffeine (CAFF) (1500 nmol) in the 1.5% formalin test. Figure 21A shows the effect on phase 1 flinches, and Figure 21B shows the effect on phase 2 flinches. Figure 21C shows the effect phase 2 biting/licking time. The empty

15   bar represents the saline control; the bar with rightward sloping lines represents the effect of caffeine (CAFF) either alone or in combination with the test drug. Values depict mean ∓ s.e.m. for n=6 per group. *=P<0.05, **=P<0.01, ***=P<0.001 compared to formalin group. NS indicates no significant difference from corresponding antidepressant group.

20

Figures 22A-B are graphs illustrating the effect of locally administered desipramine and fluoxetine on paw volume in the presence of formalin. Figure 22A shows the effect of saline (control), desipramine, or fluoxetine on paw volume following coadministration with formalin into the ipsilateral paw in the presence of

25   formalin. ●=formalin+saline; open diamond=formalin+100 nmol FLUOX; open downward pointing triangle=formalin+300 nmol DES; and open square=formalin+300 nmol FLUOX. Figure 22B shows the effect on paw swelling of desipramine or fluoxetine injected at either concentration used in Figure 22A into the contralateral hindpaw (systemic effect). Paw volumes were determined at the end of

15

the behavioral observation period. n=5-6 per group. ***=P<0.001 compared to the saline-treated group.

Figures 23A-C are a series of graphs illustrating the effects of desipramine,
5   fluoxetine, and amitriptyline as compared with saline control on paw volume when
injected into the dorsal hindpaw of the rat. Figure 23A shows the effects of 100nmol
DES (open diamond) and 300nmol DES (open square). Figure 23B shows the effects
of 10nmol FLUOX (open upward facing triangle); 30nmol FLUOX (open
downward-facing triangle), 100nmol FLUOX (open diamond), and 300nmol FLUOX
10   (open square). Figure 23C shows the effects of 100nmol (open triangle) and 300nmol
(open square) amitriptyline on dorsal paw volume. n=6 per group. *=P<0.05,
***=P<0.001 compared to the saline-injected group.

Figures 24A-B are graphs illustrating the effects of the selective 5-HT
15   receptor antagonists propranolol (5-HT$_1$), ketanserin (5-HT$_2$), tropisetron (5-HT$_{3/4}$)
and GR113808A (5-HT$_4$) on the increase in paw volume induced by local injection of
fluoxetine (FLX) (100 nmol). Figure 24A shows the percent increase in paw volume
over a three hour period of 100nmol FLX alone (●); FLX + 300nmol propranolol
(open diamond); FLX + 500nmol ketanserin (□), FLX + 300nmol tropisetron (open
20   upward-pointing triangle); and FLX + 500nmol GR113808A (open downward-
pointing triangle). Figure 24B shows the cumulative effect of the increase of Figure
24A over the same time period wherein the open bar represents FLX alone; the bar
with horizontal lines illustrates FLX+propranolol; the bar with vertical lines illustrates
FLX+ketanserin; the bar with rightward sloping lines represents FLX+tropisetron; and
25   the bar with leftward sloping lines represents FLX+GLR113808A. n=5 per group.
*=P<0.05, **=P<0.01, ***=P<0.001 compared to the fluoxetine group.

Figure 25 is a graph showing the lack of effect at dosages of 50 nmol, 150
nmol and 500 nmol ketanserin (KET), a selective 5-HT receptor antagonist, on

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formalin-evoked behaviors (phase 2 flinches) in the rat 1% formalin test. Values
depict the mean of 6 measurements per group. ● = formalin 2.5% control; □ =
propranolol;

Δ = ketanserin; open diamond = tropisetron; open hexagon = GR 113808A.

5   * = P<0.05 compared to formalin controls.

        Figure 26 is a graph showing the lack of effect of ketanserin (KET) at dosages
of 50 nmol and 500 nmol on formalin-evoked behaviors (flinches) in the rat 1%
formalin test. Values depict the mean of 6 measurements per group. ■= 1% formalin
10   control; □ = 50 nmol KET; ▲= 500 nmol KET.

        Figures 27A and B are graphs showing blockade of rat paw edema induced
by local injection of 3 nmol 5-HT by coadministration of 3 nmol or 30 nmol
ketanserin with 5-HT. Figure 27A is a graph illustrating the cumulative increase in
15   paw volume over 180 minutes. Open bars = 5-HT alone; cross-hatched bars = 5-HT +
KET (3 nmol 5-HT = left hand columns; 30 nmol 5-HT = right hand columns). Figure
27B is a graph illustrating the time course of action in terms of % increase in paw
volume over 180 minutes. ■= 5-HT 3 nmol alone; □ = 5-HT 3 nmol + KET 500
nmol; ● = 5-HT 30 nmol alone; O= 5-HT 30 nmol + KET 500 nmol.
20   ** = P<0.01, *** = P<0.001 compared to 5-HT response.

        Figures 28A-C are a series of graphs illustrating the effects of atropine,
mecamylamine and d-tubocurarine at dosages of 10, 30, 100 , 300 and 1000 nmol on
flinching and biting/licking behaviors produced by 2.5% formalin in rats. Figure 28A
25   illustrates the effects of the drugs on the number of phase 1 flinches. Figure 28B
illustrates the effects of the drugs on the number of phase 2 flinches, and Figure 28C
illustrates the effects of the drugs on phase 2 biting/licking time. (dark hexagon =
formalin 2.5%; O= amitriptyline; □ = mecamylamine; Δ = atropine (ipsilateral); dark

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triangle = atropine (contralateral); open diamond = d-tubocurarine.

* = P<0.05; ** = P<0.01; *** = P< 0.001 compared to respective formalin controls.

DETAILED DESCRIPTION OF THE INVENTION

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In accordance with the present invention, a new method has been discovered for producing local analgesia by local administration of a tricyclic, second generation or third generation antidepressant to a body site of local discomfort.

10   Examples of tricyclic antidepressants useful in the practice of this invention are those having the structure:

$$Ar_1\text{-}Z(R)\text{-}Ar_2$$
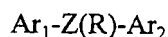
wherein Z is a 7-membered ring, optionally containing 1 or 2 biocompatible heteroatoms, or an 8-membered bicyclic ring,

15   Ar_1 and Ar_2 are optionally substituted aromatic rings fused to Z, and

R is an alkylamino or arylamino substituent.

Examples of Z as an 8-membered bicyclic ring include (2,2,2,)bicyclooctane and (3,2,1) bicyclooctane, and the like. Oxygen and nitrogen are the preferred heteroatoms for optional inclusion in Z, the central ring of the three ring structure, but

20   sulfur can also be included. R can be an alkylamino or arylamino substituent, or an N-oxide derivative thereof and generally comprises from 4 to 5 carbons, although as few as two carbons or as many as seven carbons, or even as many as 10 carbons can be contained in R. If R is an alkylamino substitutent, in some embodiments the alkylamino is a tertiary or secondary amino group. For example, R can be

25                    $-(CH_2)_3N(CH_3)_2$,

                       $-(CH_2)_3NHCH_3$,

                       $-(CH_2)_3N(CH_3)_2$,

                       $-CH_2CH(CH_3)CH_2N(CH_3)_2$,

                       $=CH(CH_2)_2N(CH_3)_2$,

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-$(CH_2)_3NHCH_3$,

=$CH(CH_2)_2NHCH_3$, and the like.
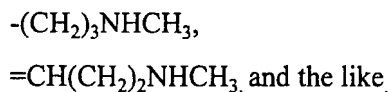
The preferred point of attachment of R to the tricyclic structure is at carbon 5, or to a
heteroatom located at position 5 in the ring structure.

5

Examples of the optional biocompatible halogen substituent for Ar_1 and/or Ar_2
are chlorine, bromine and fluorine, with chlorine being preferred. For example Ar_1
and/or Ar_2 can be substituted with one or two of the biocompatible halogen atoms.

10   Non-limiting examples of tricyclic antidepressants useful in the practice of the
present invention are clomipramine, imipramine, amitryptyline, desipramine,
nortriptyline, amoxapine, maprotiline, trimipramine, and the like, and suitable
combinations of any two or more thereof. Amitriptyline and desipramine are
presently preferred.

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Additional antidepressants contemplated for use herein include compounds
sometimes referred to as "second-generation" or "third-generation" antidepressants,
having pharmacokinetics and potency similar to those of the tricyclic antidepressants
described herein. Examples of second generation or third generation antidepressants
20   useful in the practice of the invention are those having the structure

$Ar_3(Y)-X-Ar_4(Q)$
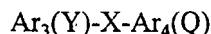
wherein Ar_3 is an N-containing heterocyclic ring, e.g., a substituted 1,2,4-
triazole or triazone,

Y is either an aryl group fused to the heterocyclic ring or one or two
25   substituents selected from alkyl, alkyloxy, arylalkyl, arylalkyloxy, aryl and heteroaryl
substituents comprising a total of about 4 to 8 carbons attached to Ar_3,

X is an alkyl group comprising 2 to 5 carbon atoms linking Ar_3 and Ar_4,

Ar_4 is a piperazine attached to X by a first nitrogen atom of Ar_4, and

19

Q is benzene ring optionally substituted with a biocompatible halogen, and attached to Q at a second nitrogen atom of Ar_4.

For example, X can contain 3 carbons, and Ar_3 can be a 1,2,4-triazone substituted at the 4 position with an arylalkyloxy substituent containing 6 to 8 carbon atoms. In an embodiment presently preferred, Y is a heteroarylalkyl substituent containing an oxygen atom.

The preferred biocompatible halogen substituents for Q are selected from chlorine, bromine and fluorine.

Representative examples of second generation or third generation antidepressants useful in the practice of the present invention are trazodone, bupropion, mirtazapine, venlafaxine, fefazodone, and the like.

In one aspect, the present invention includes compositions for local administration of tricyclic, second generation or third generation antidepressants to a subject having a site of local discomfort. Such compositions are specifically formulated to ensure substantially local, rather than systemic administration of such antidepressant(s). In addition to one or more of the antidepressants described for use herein, the compositions of the invention may comprise one or more adjuvants which facilitate delivery, such as inert carriers, penetration enhancing agents, colloidal dispersion systems.

Representative and non-limiting examples of such inert carriers can be selected from water, isopropyl alcohol, gaseous fluorocarbons, ethyl alcohol, polyvinyl pyrrolidone, propylene glycol, a fragrance, a gel-producing material, stearyl alcohol, stearic acid, spermaceti, sorbitan monooleate, methylcellulose, and suitable combinations of two or more thereof.

The topical formulation can also include one or more known penetration enhancing agents selected to have minimal allergic or irritating effect. Known penetration enhancing agents include DMSO and azacyclo compounds, such as those

5   disclosed in U.S. Patent Nos. 4,755,535, 4,801,586, 4,808,414, an 4,920,101, which are incorporated herein, each in its entirety. No more of a penetration enhancing agent should be included in such compositions than is consistent with the goal of attaining substantially local delivery, rather than systemic delivery, of the tricyclic, second generation or third generation antidepressant(s). Those of skill in the art will

10   be able to select a suitable penetration enhancing agent useful for the particular degree of penetration desired.

In another embodiment, the invention composition is formulated for local injection. In such an embodiment, the composition generally comprises a

15   physiologically compatible saline solution and may optionally be encapsulated in a slow release delivery vehicle suitable for local injection, such as a colloidal dispersion system or in polymer stabilized crystals. Colloidal dispersion systems include nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system

20   of this invention is a liposome or microsphere. Liposomes are artificial membrane vesicles which are useful as slow release delivery vehicles when injected or implanted, or when contained within a topical preparation. One type of microspheres suitable for encapsulating drugs for local injection is poly(D,L)lactide microspheres, as described in D. Fletcher, *Anesth. Analg.* 84:90-94, 1997.

25

The invention compositions may be formulated for topical administration, for instance as a cream, a lotion, a gel, an ointment, a spray, or an aerosol. Tricyclic, second generation and third generation antidepressants are quite lipid soluble themselves and, readily cross lipid barriers to gain access to central nervous system

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sites. This same property enables them to cross the perineurial barrier surrounding

peripheral nerve endings (this barrier limits access of some agents to such sites, I.

Antonijevic et al., Perineurial defect and peripheral opioid analgesia in inflammation,

J. Neurosci 15 (1995) 165-172), giving them ready access to the peripheral nerve

5   terminal and adjacent sites. Topical administration can also be effected by delivering

the invention compositions via a slow release "patch." Therefore, in yet another

embodiment, the composition further includes a patch delivery system having the

antidepressant sequestered for slow release upon application to skin or mucous

membrane.

10

In another aspect of the invention, there are provided methods for producing

local analgesia in a subject, such as a mammal, by locally administering an effective

amount of a tricyclic, second generation or third generation antidepressant(s) to a site

of local discomfort. The term "local administration" includes any method of local

15   administration known in the art. The antidepressant(s) used in the practice of the

invention can be injected locally, for example, by subcutaneous or intra-articular

injection. Alternatively, the tricyclic, second generation or third generation

antidepressant(s) can be applied topically by applying a cream ointment, spray, or gel

comprising the any antidepressant contemplated for use herein to an area of skin

20   adjacent to a site of pain. Additional routes of local administration contemplated

herein include topical application, local injection or subcutaneous implantation of the

antidepressant encapsulated in a slow release vehicle, such as a liposome or

microsphere.

25   The term "effective amount" means the quantity of a compound according to

the invention necessary to prevent, to cure, or at least partially arrest a symptom of

local pain or discomfort in a subject. A subject is any mammal, preferably a human.

Amounts effective for creating a substantially local therapeutic effect will, of course,

depend on the severity of the disease causing the painful condition, and the weight

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and general state of the subject. Typically, animal models, such as those described in the Background and Examples herein, may be used to determine suitable dosages to be used to achieve substantially local delivery of the tricyclic, second generation or third generation antidepressant(s). A recent pain scale developed by Galer et al.

5   (Development and preliminary validation of a pain measure specific to neuropathic pain: The Neuropathic Pain Scale, Neurology 48 (1997) 332-338), which uses terminology specific for neuropathic pain, should be better able to delineate the symptoms within the syndrome. In addition, various general considerations taken into account in determining the "therapeutically effective amount" are known to those

10   of skill in the art and are described, e.g., in Gilman et al., eds., Goodman And Gilman's: The Pharmacological Bases of Therapeutics, 8th ed., Pergamon Press, 1990; and Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pa., 1990, each of which is herein incorporated by reference.

15   The compositions and methods of the invention are particularly suited to producing local analgesia for painful symptoms known in the art as "neuropathic pain." As used herein the term "neuropathic pain" refers to pain syndromes known to be neuropathic (i.e., due to lesions or dysfunction in the nervous system) including certain relatively generalized syndromes, such as peripheral neuropathy, phantom

20   pain, reflex sympathetic dystrophy, causalgia, central pain, syringomyelia, painful scar, and the like. Certain relatively localized syndromes are also considered to be neuropathic. Among these are specific neuralgias at any location of the body, head or face; diabetic, alcoholic, metabolic or inflammatory neuropathies; post herpetic neuralgias; post traumatic and post endodontic odontalgia; thoracic outlet syndrome;

25   cervical, thoracic, or lumbar radiculopathies with nerve compression; cancer with nerve invasion; post traumatic avulsion injuries; post mastectomy pain, post thoracotomy pain; post spinal cord injury pain; post stroke pain; abdominal cutaneous nerve entrapments; primary tumors of neural tissues; and arachnoiditis, and the like.

Other pain syndromes believed to have a neuropathic component are stump pain, fibromyalgia, regional sprains or strains (crushing injury), myofascial pain, psoriatic arthropathy, polyarteritis nodosa, osteomyelitis, burns involving nerve damage, AIDS related pain syndromes, and connective tissue disorders, such as

5   systemic lupus erythematosis, systemic sclerosis, polymyositis, and dermatomyositis, and the like.

The compositions and methods of the invention are also particularly suited to producing local analgesia for symptoms of localized pain and discomfort caused by

10   inflammatory conditions. Inflammatory conditions that can be treated in the practice of the invention include conditions of acute inflammation (e.g. trauma, surgery and infection) or chronic inflammation (e.g., arthritis and gout).

The relative high concentrations of drugs attainable by local administration,

15   coupled with a lesser incidence of the side effects characteristic of systemic absorption, produce particular benefits in treatment of discomfort associated with inflammatory or neuropathic pain conditions using the compositions and methods of this invention.

20   A rat spinal nerve ligation (SNL) model was used to determine the effect of tricyclic, second generation or third generation antidepressant(s) against neuropathic pain as described in full in the Examples herein. In this model, locally administered amitriptyline and desipramine, tricyclic antidepressants, were shown to be effective in alleviating thermal hyperalgesia. The local anti-hyperalgesic effects of amitriptyline

25   and desipramine administered to a nerve-damaged hindpaw were observed without a concomitant analgesic effect on the contralateral paw. The maximal effect of locally administered amitriptyline was equal in magnitude to that of systemic injection, but alleviated manifestations of neuropathic pain over a longer time period than did systemic administration of the drug. This local effect produced no change in the

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thermal threshold of the contralateral paw or the paw of naive animals following local
administration. Further, the local nature of this effect was verified when injections
into the contralateral paw were found to have no significant effect on ipsilateral
response latencies.

5

In contrast to the beneficial actions of locally administered amitriptyline
against thermal hyperalgesia, amitriptyline was observed to be ineffective in the
treatment of nerve injury-induced mechanical allodynia. There may even be a slight
untoward effect following spinal administration of amitriptyline (60 μg) that became
10   statistically significant when the cumulative change in the maximum possible effect
(Δ MPE) was analyzed (Figure 6C). Increasing the spinal dose of amitriptyline to
90 μg, however, caused a significant sedative effect so that the animals were flaccid
in the testing apparatus, a result that hindered their ability to respond to the stimuli.
An interesting observation with the use of amitriptyline in the allodynia test was the
15   decrease in response threshold observed in the contralateral paw (Figs. 5A-C, 6A-C,
and Figures 7A-C), an effect referred to herein as a "hyperaesthetic tactile response."
While the hyperaesthetic response threshold was similar to that of the nerve injured
paw qualitatively, the observed behavioral response (a brisk withdrawal) differed from
that exhibited by a normal neuropathic pain response (i.e., biting, licking, and
20   guarding). The results of this study indicate that amitriptyline preferentially alleviates
thermal hyperalgesia through all routes of administration, but has no effect on
allodynia.

These findings suggest that the pathophysiology of thermal hyperalgesia and
25   tactile allodynia are maintained through distinct mechanisms, which has also been
suggested by the differential profile of other drugs like dextromethorphan on these
two endpoints (M. Tal and G.J.Bennett (1994) *Neuroreport* 5:1438-1440). Therefore,
the efficacy of tricyclic antidepressants, such as amitriptyline in a patient may be
determined by which of the neuropathic symptoms is the most strongly expressed.

25

For example, in a patient presenting with robust allodynic like symptoms, amitriptyline and antidepressants with similar activity may not make any perceived difference in the pain. However, if the dominant complaint is one that can be characterized as hyperalgesia, amitriptyline and drugs with similar activity may

5   provide relief.

The experiments upon which this invention is based also demonstrate a local peripheral antinociceptive action for antidepressants, such as tricyclic, second generation and third generation antidepressants, against inflammation. In the rat

10   formalin test, a well known animal model for inflammatory conditions, the antinociceptive action is shown by reduction in pain responses (both flinching behaviors and biting/licking time) caused by injection of formalin into a rat paw. The local nature of this action is verified in the animal model by the lack of effect of amitriptyline injected into the contralateral hindpaw. The antinociceptive effect is

15   seen at locally injected doses of amitriptyline up to 100 nmol, which corresponds to a systemic dose of 0.22 mg/kg (for a 142 g rat). In the 2.5% formalin test, amitriptyline, desipramine and fluoxetine 10-300 nmol produced a dose-related reduction in phase 2 (16-60 min) flinching and in biting/licking behaviors when coadministered with the inflammatory irritant, formalin. Phase 1 flinch behaviors (0-

20   12 min) were reduced at the highest dose. This action was locally mediated, as it was not seen when the antidepressants (100, 300 nmol) were injected into the contralateral hindpaw. The local action of desipramine and fluoxetine was partially altered by coadministration of caffeine 1500 nmol, but the action of amitriptyline was reversed by caffeine.

25

The peripheral action of amitriptyline involves an interaction with endogenous adenosine systems as shown by the Examples herein. A similar peripheral antinociceptive action of NH_2dAD, an inhibitor of adenosine kinase, which also acts

by changing the extracellular availability of adenosine, is shown in the rat formalin test.

The local effect of locally administered tricyclic, second generation or third

5   generation antidepressants as demonstrated in the formalin test is quite robust, with phase 1 and phase 2 flinching behaviors and the total biting/licking time being suppressed markedly.

While the mechanism of action of the antidepressants does not form a part of

10   this invention, it has been determined that the peripheral antinociceptive action of amitriptyline involves endogenous adenosine with a subsequent activation of adenosine A_1 receptors on sensory nerve terminals (reviewed Sawynok J. (1997) In: *Purinergic Approaches to Experimental Therapeutics* (Ed. Jacobsen K., Jarvis M.) Wiley-Liss Inc., New York, 495) This observation is based on the ability of caffeine,

15   a non-selective adenosine A_1 and A_2 receptor antagonist, and CPT, a selective adenosine A_1 receptor antagonist, to inhibit the action of amitriptyline on phase 2 flinching responses and biting/licking time. Phase 1 responses were unaffected by caffeine, and may result from another mechanism.

20   The studies upon which this invention is based also demonstrate that an inhibitor of adenosine kinase (NH_2dAD) can augment the local action of amitriptyline. Adenosine kinase is an intracellular enzyme (reviewed J.D.Geiger et al., Regulators of endogenous adenosine levels as therapeutic agents in: Purinergic approaches in *Experimental Therapeutics*, Ed. Jacobsen, K.A. and Jarvis, M.F., Wiley-Liss Inc.,

25   New York (1997) 55-84) and its inhibition would result in an intracellular accumulation of adenosine and an efflux of adenosine from the cell along a concentration gradient This effect would enhance the extracellular adenosine available for uptake and the interaction with amitriptyline. Inhibition of adenosine

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deaminase with 2'-deoxycoformycin did not produce the same response, and this is
generally consistent with the relative intrinsic effects expressed by these two agents.

5   It is important to note that in the experiments described herein all of these
influences on adenosine availability are superimposed on an inflammatory stimulus
(formalin). Endogenous adenosine is released peripherally by formalin (Doak GJ,
Sawynok J. (1995) *Eur J Pharmacol* 281: 311), as well as by inflammatory processes
in general (Cronstein, 1994). Thus, the stimulus generates the substrate (adenosine
and its uptake) with which amitriptyline interacts. The lack of, or more limited, effect
10   of amitriptyline observed at lower concentrations of formalin inflammatory model
(Figures 10A-C) may well be due to an inadequate release of endogenous adenosine at
the lower stimulus intensity.

      Similarly, the experiments on which this invention is based have shown that
15   caffeine and CPT were without an intrinsic effect at 1.5% formalin, or slightly
reduced the effect of formalin at 2.5% (cf. Figures 11A-C, 12A-B and 13A-B). The
lack of effect of CPT indicates that the amount of endogenous adenosine generated by
the formalin was insufficient to produce antinociception; such a response only
occurred when amitriptyline was present and inhibited adenosine uptake. The modest
20   effect of caffeine at 2.5% formalin may reflect a slight predominance of adenosine A_2
receptor activation in this experiment. Caffeine has been shown to lack a peripheral
intrinsic effect due to blockade of opposing influences of adenosine A_1 and A_2
receptors (Doak G.J., Sawynok J. (1995, supra).

25   In the spinal nerve ligation model, amitriptyline (100 nmol) and desipramine
(100 nmol), but not fluoxetine (100 nmol), produced a local and complete
antihyperalgesic action in the hindpaw corresponding to the ligated side when
thresholds were determined using a thermal paw stimulator. Desipramine and
amitriptyline, at 100 and 300 nmol, which are maximally effective doses, produced a

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slight increase in paw volume, but fluoxetine produced a robust and sustained increase in paw volume over the complete dosage range (10-300 nmol). No agent significantly altered the degree of paw swelling produced by formalin in the inflammatory pain model when coinjected with the formalin. The increase in paw volume produced by

5   fluoxetine was inhibited by ketanserin, a selective 5-HT$_2$ receptor antagonist, but not by other selective 5-HT receptor antagonists. The pronounced local pain alleviating actions in the absence of marked changes in paw volume produced by the tricyclic antidepressants desipramine and amitriptyline in the formalin test and the spinal nerve ligation model indicate that tricyclic, second generation or third generation

10 antidepressant(s) can be used to produce local analgesia, for example in inflammatory and neuropathic pain states.

The invention will now be described in greater detail by reference to the following non-limiting examples.

15

EXAMPLE 1

Experiments were conducted on male Sprague-Dawley rats (100-120 gm) (Charles River, Quebec, Canada). Animals were housed in pairs, maintained on a

20 12:12 hour light-dark cycle at 22°∓1°C and given ad-libitum access to food and water.

A. Preparation of the spinal nerve ligation animal model

Surgery on male rats was performed using aseptic technique under a dissection

25 microscope and with adherence to the guidelines of the IASP on animal experimentation in pain research. The neuropathic condition was created by a tight ligation of the dorsal roots of lumbar nerves L-5 and L-6. Sprague Dawley rats (100-125 gm) were anaesthetized and surgically maintained using Halothane (1.5-2.0%). The animals were surgically clipped in the dorsal pelvic area. Antibacterial eye drops

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were applied to the eyes to prevent drying through the course of the surgical procedure. The animals were placed on a water heated pad (37±0.5°C). The area for surgical incision was cleansed and sterilized with ethyl alcohol and iodine. A 3 cm incision was made along the dorsal midline with the position of the iliac crest

5   indicating the incision midline. A stab incision was made on the left side of the vertebral column at the level of the sacrum. The ligament attachments to the spinal cord were cut 2 cm rostral to the stab incision. Blunt dissection was used to further disconnect and separate the remaining tissue from the spinal cord. One retractor was placed at the L-4 vertebrae and a second retractor was used to counter retract the

10   muscle away from the sacro-iliac crest. Blunt dissection was used to clear the sacro-iliac crest, the L-6/S-1 articular (facet) joint, and the transverse process of L-6. The facet was nipped off with rongeurs. A third retractor was used to retract the muscle away from the L-6 transverse process.

15   Under the dissecting microscope and using jewelers forceps, the connective tissue between the L-6 transverse process and the sacro-iliac rim was removed. Rongeurs were used to slowly nip away the transverse process of L-6 and expose the area for access to the L-5/L-6 nerve bundle. Using a glass probe the L-4/L-5 nerve bundle was hooked and manipulated to isolate the L-5 nerve. Once free of the L-4

20   nerve, the L-5 nerve was tightly ligated using 6-0 silk so that the nerve bulged on both sides of the ligature. The suture ends were trimmed and the nerve was allowed to fall back into its resting position. Again using the glass probe, the L-6 nerve was hooked from under the sacro-iliac rim, tightly ligated (6-0 silk), and placed back in position under the sacro-iliac rim. All damaged tissue was debrided, and all blood swabbed

25   clear of the incision site. The muscle and tissue was gently rolled back into place to prevent any subdermal air pockets. The fascia over the muscle, and subsequently the skin was sutured shut (both using 3-0 Vicryl).

Post-operatively, the animals were placed in a recovery box with a heat lamp

for observation. Following a successful surgery, the animals were fully ambulatory

with no indications of spontaneous pain (licking, biting, or autonomy of the

neuropathic foot, or vocalizations). If the animals presented initially with a foot drop

5   of the neuropathic paw, that disappeared within 1-2 days post-operatively. Any

animals that presented with locomotor deficits or symptoms of spontaneous pain

2 days post-operatively, were promptly euthanised with an overdose of halothane.

B. Behavioral Testing

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All behavioral tests were conducted between 08:00 and 14:00 daily, and

occurred at 7 ,12, 17, and 22 days following nerve injury. An exception to this was

the cannulated animals, which were tested at 7 and 12 days after cannulation (14 and

19 days after nerve injury respectively). Following a recovery period of 7 days,

15   animals were moved from the vivarium, weighed, and acclimatized to the testing

room for 40 minutes. After this initial period the animals were placed in the testing

apparatus for 30-40 minutes or until exploratory behavior ceased.

B.1. Establishment and maintenance of neuropathic pain for the testing

20   **periods**

To determine that peripheral mononeuropathy resulted from ligation of L5 and

L6 spinal nerves tests of static mechanical allodynia and thermal hyperalgesia were

conducted.

25

B.2. Testing for Thermal Hyperalgesia

Surgically induced thermal hyperalgesia was assessed using a Paw Thermal

Stimulator System. The animals were allowed two acclimatization periods prior to

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the testing (30 minutes post removal from housing facility; 20 minutes in individual

testing chambers). Rats were placed in pairs in a plexiglass box on top of the

temperature maintained glass surface (30 ± 0.1°C) of the stimulator. After the initial

acclimatization period, rats were tested for baseline withdrawal latencies (seconds) of

5   both paws once every 20 minutes until three stable baselines were achieved. The

animals were then returned to their cages for 30-40 minutes prior to drug

administration and given ad-libitum access to food and water. Following drug

administration, the animals were returned to the testing boxes for testing.

10   **B.3. Static Mechanical Allodynia**

Following weighing and testing room acclimatization, rats were placed in an

elevated plexiglass container with a wire mesh bottom to allow access to the ventral

surface of the hind paws. Rats were further acclimated to the testing chambers and

15   tested for experimental baseline (two tests in one hour) using Semmes-Weinstein

monofilaments (Stoelting Comp., Wood Dale IL.). The 50% withdrawal threshold

was determined using the Dixons up-down method (Chaplan et al., 1994). Briefly,

filaments were applied to the ventral surface of the paw starting with the 4.31 filament

(2.04 gm) and the response noted. Following a positive response (paw withdrawal,

20   with characteristic pain behavior), a lower filament was then applied. If no response

was shown, the weight of the subsequent filament applied was increased. The 50%

withdrawal threshold was then determined from the tabular value for the pattern of

responses (k), the final monofilament value $(X_f$, in log units), and interpolated using

the formula:

25   50% g threshold $= (10^{[X_f + k\delta]}) / 10{,}000$

where δ is the mean difference between stimuli. Following the determination of

baseline values, the rats were returned to their original cages and left undisturbed with

ad-libitum access to food and water for 30-40 minutes. After drug administration, the

animals were again placed in the testing chambers and monitored for the appropriate

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behavioral periods depending on the route of drug administration. The results of these tests are shown in Figure 1.

B.4. Data Analysis

5

For each of the experiments, raw data for response thresholds of both paws in each animal were recorded and entered into a spreadsheet (Microsoft Excel 5.0). The data was then normalized for each animal as Maximum Possible Effect (MPE) in terms of reversal of the neuropathic symptom being tested (MPE (anti-hyperalgesia or

10   antiallodynia)) or in terms of the analgesic effect of the contralateral paw (MPE (analgesia)). These values were calculated as follows:

$$MPE \text{ (anti-neuropathic)} = (PDR-IBR)/(CBR-IBR),$$

wherein PDR is the post drug response of the ipsilateral paw, IBR is the ipsilateral paw baseline response, and CBR is the contralateral paw baseline response.

15   Similarly, the MPE (analgesia)was calculated as follows:

$$MPE \text{ (analgesic)} = (PDR - CBR)/(cutoff-CBR),$$

wherein cutoff was 20 seconds from the thermal stimulus and 15 g for tactile allodynia, and PDR is the post drug withdrawal threshold of the contralateral paw. Accordingly, the individual values reported or depicted are the MPE ± SEM. The

20   time course of the drug effect is also depicted as the cumulative change in the MPE (Δ MPE) for each treatment and route of administration. This value was calculated as the sum of the individual MPE values through the time course (Σ_n), assuming an MPE value of 0 for the baseline values (not shown in figures). The data was statistically analyzed using a one way ANOVA for repeated measures with a pairwise comparison

25   (Dunnetts post hoc analysis). To compare the ΔMPE values of multiple treatment groups a one-way ANOVA with a pairwise comparison (Student-Neuman-Keuls post hoc analysis) was used. When comparing the means at individual time points or in the ΔMPE values, a student's T-test was used with a Student-Neuman-Keuls post hoc correction. For all tests, a P value less than .05 (P<.05) was considered significant.

These tests showed that spinal ligation resulted in neuropathic pain in rats with presenting symptoms of static mechanical allodynia and thermal hyperalgesia, and the condition was maintained throughout the testing periods (days 7, 12, 17, 22). As

5   shown by the data summarized in Figure 1, the threshold values for thermal hyperalgesia were 7.98 ± 0.48 sec for the ipsilateral paw as compared to 10.35 ± 0.63 sec for the contralateral paw. Allodynia was manifested as 50% withdrawal threshold of 2.20 ± 0.28 g for the ipsilateral paw and 13.37 ± 0.37 g for the contralateral paw. Threshold values remained relatively stable with no statistically

10   significant difference in baseline values for either the ipsilateral or the contralateral paw on each of the subsequent testing days.

B5. Drug Treatment

15   Tests were conducted to determine the antinociceptive effect of the tricyclic antidepressant amitriptyline in the ligated rat model. All drugs treatments were blinded. In both of the behavioral tests, amitriptyline (Sigma Chemical Co., St. Louis, MO) or saline was administered either systemically (by intraperitoneal (i.p.) injection), spinally (through an implanted intrathecal cannulas (i.t.)), or locally (by

20   subcutaneous injection (s.c.) in the dorsal surface of the paw). Drugs were injected in volume of 5 ml/kg for systemic injections, and a total volume of 20 μl (10 μl drug + 10 μl saline flush) for intrathecal injections. For local injections, the animals were briefly anaesthetized with halothane, and the solution was administered s.c. to the dorsal surface of the ipsilateral or contralateral paw in a volume of 50 μl.

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C1. Effect of systemic amitriptyline on nerve injury-induced thermal hyperalgesia

Systemic administration to nerve-injured rats of amitriptyline at doses of 1, 3
and 10 mg/kg of body weight by ip. injection produced an anti-hyperalgesic effect on
the thermal threshold of the nerve-injured paw, as shown by the data summarized on
Figures 2A-C. This effect was maximal at 10 mg/kg; reaching an almost complete
reversal of thermal hyperalgesia (MPE =0.94 $\pm$ -0.17) 60-80 minutes after injection
(Figure 2A). A slight but not statistically significant elevation in threshold was
observed in the contralateral paw for the 10 mg/kg dose (Figure 2B). Comparison of
the ΔMPE values for the ipsilateral paw (Figure 2C), shows a statistically significant
antinociceptive effect of 10 mg/kg for amitriptyline as compared to saline controls, as
well as for 1 mg/kg and 3 mg/kg doses. While both 1 mg/kg and 3 mg/kg doses
produced results significantly different from those of the saline controls, they were not
significantly different from each other. A slight sedative effect of the 10 mg/kg dose
was observed in a few of the rats, but none was observed at other doses. This effect
did not impact the ability of the animals to respond to the stimulus, as the vigor of the
response at threshold was similar to that observed during baseline determinations.

C2. Intrathecal Cannulations

In animals used to study the effects of spinal administration of amitriptyline,
intrathecal cannulas were implanted 7 days following nerve injury. For the
implantation of intrathecal cannulas for the spinal delivery of drugs, rats were
anaesthetized with halothane and mounted in the earbars of a stereotaxic apparatus.
The cisternal membrane at the base of the skull was exposed, a small incision made,
and a cannula (7.5 cm of PE-10 tubing) inserted into the subarachnoid space. The
cannula was filled with saline and the external end was occluded with a small wire
plug. Rats were injected with Penlong XL^R (0.15ml i.m.) and lactated Ringers

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solution (10 ml s.c.) while still anaesthetized. On the day of testing, rats were

accommodated in a plastic container for the intrathecal injection (in a 10µl volume

followed by 10µl saline to flush the cannula).

5   **C3. Effect of spinal amitriptyline on nerve-injury induced thermal**

hyperalgesia

           The intrathecal injection of amitriptyline (60 µg) resulted in a partial reversal

of the thermal hyperalgesia of the ipsilateral paw in nerve injured rats that was evident

10   20 minutes after injection and lasted for the 60 minute testing period, as shown by the

data in Figures 3A-C. Because a 30 µg dose was ineffective in the allodynia test, and

90 µg was highly sedative, these doses were not used for testing of the thermal

hyperalgesic effect. While values for the individual time point are not significantly

different on their own (Figure 3A), the ∆MPE values for saline and amitriptyline

15   (60 µg) were significantly different (Figure 3C). No significant difference was

observed, in the contralateral paw withdrawal thresholds, at either single time points

or in comparison of the ∆MPE values (Figures 3B and 3C).

           C4. Effect of local injection of amitriptyline on nerve injury-induced

20   **thermal hyperalgesia**

           When injected locally into the neuropathic paw, amitriptyline at a dose of 100

nmol, which produces a local antinociceptive effect in the rat formalin test as

described in Example 2 herein (Figure 8), had an immediate statistically significant

25   antinociceptive effect, almost completely reversing the thermal hyperalgesia in nerve-

injured rats (Figure 4A). The MPE values remained significantly different from those

of saline for the first 120 minutes. A contralateral local injection of amitriptyline

(100 nmol) failed to show any significant effect on the withdrawal latency of the

ipsilateral paw (Figure. 4A), a result that indicates a lack of systemic effect. No local

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effect of the injected paw was observed either for the control rats injected with saline (ipsilateral paw) or for animals injected with amitriptyline (100 nmol) in the contralateral paw.

5   There was also no significant alteration in the withdrawal thresholds of naive animals after local injection of amitriptyline 100 nmol (n=4; data not shown). In addition, neither the ipsilateral nor contralateral local injections showed any significant effect on the contralateral paw withdrawal latency (Figure 4B). Comparison of the ΔMPE values revealed a statistically significant difference

10   resulting from locally injected amitriptyline on thermal hyperalgesia as compared to both the saline control and the contralateral injection control (Figure 4C).

C5. Effect of systemic amitriptyline on nerve injury-induced mechanical allodynia

15

Amitriptyline was systemically administered to nerve injured rats at doses of 1.5, 5.0, and 10.0 mg/kg of body weight. No significant effect on nerve injury induced allodynia in the ipsilateral paw was observed at any of the doses (Figure 5A). Since 10 mg/kg was slightly sedative in some rats, a higher dose was not used. In the

20   contralateral paw, all doses of amitriptyline caused a hyperaesthetic response resulting in a reduced MPE (analgesia) value ranging from -0.4 to -0.8 (Figure 5B). The Δ MPE values for each dose differed significantly from saline (Figure 5C). Qualitatively, the hyperaesthetic response was observed as a brisk withdrawal to the filament with no overt signs of typical neuropathic pain responses such as biting,

25   licking, vocalization or guarding of the paw. Although the values of the response threshold in the non-injured paws were very similar to those in the injured paws, the responses were not categorized as 'neuropathic pain responses'. Threshold testing 24 hours after drug administration showed that both the ipsilateral and contralateral baselines returned to predrug values (data not shown).

C6. Effect of spinal amitriptyline on nerve injury-induced mechanical allodynia

5   Amitriptyline was also administered spinally to test its antinociceptive effect
against nerve injury-induced mechanical allodynia in the ipsilateral paw, At doses of
30, 60, and 90 μg administered spinally, amitriptyline failed to have a significant
effect on allodynia in the ipsilateral paw (Figures 5A and 6C). However a comparison
of the ΔMPE for the three groups revealed the 60 μg group to be significantly

10   different from the 30 μg and 90 μg groups, as well as from the saline group
(Figure. 6C). Spinally administered amitriptyline (60 μg) caused a hyperaesthetic
response in the contralateral paw (Figures 6B and 6C) that was similar in magnitude
to that observed following systemic administration. While 90 μg dose (n=3) appeared
not to facilitate the hyperaesthetic response, this result was probably due largely to the

15   pronounced sedative effect of the drug at this dose, which interfered with accurate
determination of the withdrawal threshold.

C7. Effect of local amitriptyline on nerve-injury-induced mechanical allodynia

20

        Amitriptyline was also injected locally to test the local effect on nerve-injury
induced mechanical allodynia. Local injection of amitriptyline (100 nmol) failed to
show any significant effect on the withdrawal response of the nerve injured paw to
tactile stimulation (Figures 7A and 7C). Local injection of amitriptyline into the

25   nerve injured paw did, however, cause a reduction in the response threshold of the
contralateral paw. This effect also occurred after direct local injection of amitriptyline
(100 nmol) into the non-injured contralateral paw (Figure 7B). The response to
locally administered drug in the contralateral paw was observed to be significantly
different from the response to saline, but not different from the response following

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injection into the non-injured paw at individual time points (Figure 7B) and in
comparison of the ΔMPEs (Figure 7C).

EXAMPLE 2

5

Experiments were performed using male Sprague-Dawley rats (120-160 g;
group mean 142∓4 g) (Charles River, Quebec, Canada). Rats were housed in pairs
and maintained on a 12:12 h light/dark cycle at 22°∓1°C. Food and water were freely
available.

10

The test drugs were amitriptyline, caffeine, 5'-amino-5'-deoxyadenosine
(NH$_2$dAD), phentolamine HCl, dimethylsulfoxide (DMSO) (Sigma Chemical Co., St.
Louis, MO), 8-cyclopentyl-1,3-dimethylxanthine (CPT),
N^{6}benzyl-N-ethylcarboxamide adenosine (N^{6}benzyl-NECA), MK-801 hydrogen

15   maleate, naloxone HCl (Research Biochemicals Inc., Natick, NJ),
2'-Deoxycoformycin (Parke Davis Pharmaceutical Research Division, Ann Arbor,
MI). Formalin (37% formaldehyde) (British Drug Houses, Toronto, Ontario) was the
control irritant. All drugs except for CPT were dissolved in saline and diluted to the
appropriate dose with formalin. CPT was dissolved in a final concentration of 15%

20   DMSO, and appropriate control groups were included.

A. The Formalin Test

On the day of testing, rats were placed in a 28x28x28 cm plexiglass

25   observation chamber for an initial 20 min to allow familiarization with surroundings.
The indicated doses of formalin (0.5-2.5%) and formalin/drug combinations were
injected s.c. in a volume of 50μl into the dorsal hindpaw of the rat. To establish
whether inhibition of pain response was due to a systemic rather than a local effect,
the test drug was administered into the contralateral paw. For injections into the

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contralateral paw, the drug was injected immediately before the formalin in the other

paw. Following injections, rats were returned to the observation chamber, and

flinching behaviors (lifting, shaking or rippling of the haunch) were monitored over

two time periods, phase 1 (0-12 minutes following administration) and phase 2 (16-60

5   minutes following administration). Biting/licking time was also monitored. Flinches,

which were rapid events, were recorded as discrete episodes, while the time spent

biting or licking was recorded cumulatively. Two rats in adjacent chambers were

observed at a time, with observations taking place in alternating 2 min bits. Recorded

episodes and times were not corrected, with the result that values obtained represent

10   about half of the total behaviors expressed.

Data was analyzed using analysis of variance followed by the Student-

Neuman-Keuls test for multiple groups, or the Student t-test for two groups.

15   **B. Local Antinociceptive Action of Amitriptyline**

To test the antinociceptive effect of locally administered antidepressant,

amitriptyline was coadministered at doses of 10, 30, and 100 nmol with 2.5%

formalin. A dose-dependent reduction in the expression of flinching behaviors both in

20   phase 1 and phase 2 following injection was observed. The time course for these

effects is presented in Figure 8A, and the cumulative scores for the two phases are

presented in Figures 9A and 9B. A similar dose-related inhibition in biting/licking

behaviors was obtained (Figures 8B and 9C); however, there was no distinct phase 1

effect observed in biting/licking behaviors (Figure 8B). The inhibitory effect of

25   amitriptyline on both parameters was not observed following administration of

amitriptyline into the contralateral hindpaw (Figures 9A-B). The absence of

inhibitory effect on the contralateral hindpaw indicates that the antinociceptive action

of amitriptyline is a local effect.

40

Inhibition of pain was seen most clearly at higher concentrations of formalin. For example at 0.75% formalin, the effect was seen only with flinching behaviors, while at 0.5%, it was not seen at all (Figure 3).

5   ## C. Involvement of Adenosine in the Local Antinociceptive Action of Amitriptyline

To examine involvement of adenosine in the antinociceptive action of amitriptyline, caffeine, a non-selective adenosine antagonist, was coadministered at

10   doses of 500 and 1500 nmol with amitriptyline. A significant reversal of the antinociceptive action of amitriptyline was observed in phase 2 flinches and total biting/licking time, but phase 1 flinches were not affected (Figures 11A-C). Although there was only a partial reversal of amitriptyline action at 2.5% formalin caused by caffeine, caffeine clearly reversed the local effects of adenosine. Therefore, it was

15   speculated that the expression of adenosine may depend on the intensity of the pain stimulant. To test this hypothesis, some experiments were undertaken using a lower concentration of formalin. Amitriptyline at 100 nmol concentration reduced phase 2 flinches to formalin 1.5% to a lesser extent than in the 2.5% formalin test, but caffeine produced a complete reversal of the antinociceptive action of amitriptyline

20   (Figure 13A). However, at a higher dose of amitriptyline (300 nmol), which produced a comparable extent of inhibition as did 100 nmol with 2.5% formalin, the degree of reversal by caffeine was still only partial (Figure 13A).

CPT, a selective adenosine A_1 receptor antagonist, coadministered at a dose of

25   150nmol with 100 nmol amitriptyline produced a similar degree of reversal of the action of amitriptyline as did caffeine in the 1.5% formalin test (Figure 13B). At 1.5% formalin, the effect of amitriptyline on phase 1 flinches was not reversed in any experiment, while the effect on biting/licking was reversed only with the caffeine at 100 nmol amitriptyline (data not shown). Caffeine and CPT did not produce an

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intrinsic effect in the 1.5% formalin test (Figure 13A-B), but caffeine produced a slight reduction in flinches at 2.5% formalin concentration as can be seen by comparing the data of (Figures 11A-C and Figures 12A-B).

5   Coadministration of NH_2dAD, an inhibitor of adenosine kinase, at a dose of 100 nmol augmented the action of a partially effective dose of amitriptyline on phase 2 flinches in a 2.5% formalin test (Figure 14), but was without effect on phase 1 flinches and biting/licking time (data not shown). This dose of NH_2dAD is without intrinsic effect on formalin behaviors at this concentration of formalin.

10

D. Involvement of Other Mechanisms in the Local Antinociceptive Action of Amitriptyline

Amitriptyline is known to exert a number of pharmacological effects.
15   Experiments were conducted to test for potential involvement of interactions with excitatory amino acid receptors, biogenic amines (noradrenaline, 5-hydroxytryptamine or 5-HT, histamine) and endogenous opioids by determining effects of respective receptor antagonists. The coadministration of the non-competitive NMDA receptor antagonist, MK-801 at doses of 20 and 100 nmol with formalin produced a dose-
20   dependent reduction in phase 2 flinching behaviors and biting/licking time (Figures 15A-B), with the latter behavior exhibiting a greater sensitivity to inhibition. No significant effects were seen on phase 1 flinching behaviors (data not shown). This action of MK-801 is not locally mediated, as shown by the identical effect seen following administration of the MK-801 into the contralateral hindpaw
25   (Figures 15A-B). These results indicate that MK-801 acts by a systemic route. Injection of 100 nmol (but not 20 nmol) into both the ipsilateral and contralateral hindpaws also produces a behavioral hyperactivity during the observation interval. Lower doses of MK-801 (0.03 and 0.3 nmol) coadministered with formalin had no effect on any parameter (n=4 each, data not shown).

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Coadministration of phentolamine (PTA), a non-selective-adrenoreceptor antagonist, had no significant effect on the action of amitriptyline at 100 nmol, but coadministration of 500 nmol augmented its action (Figure 16A). This potentiation is

5   due to an additive action of an intrinsic effect of phentolamine with that of amitriptyline, as phentolamine alone at this dose inhibited the action of formalin (Figure 16A). An identical pattern of interactions was seen with the phase 2 biting/licking time, but no significant effects were seen with phentolamine on phase 1 flinching (data not shown).

10

To examine a potential local antihistamine or anti-serotonergic action for amitriptyline, tests were designed to determine whether amitriptyline could alter the flinching behaviors produced by N^6-benzyl NECA, an adenosine A_3 receptor agonist which produces intrinsic nociceptive behaviors by releasing histamine and 5-HT from

15   mast cells. In these tests, 100 nmol amitriptyline did not alter the number of flinches generated by 5 nmol N^6-benzyl-NECA (25.5∓6.3 flinches in the absence, and 27.8∓4.3 flinches in the presence of amitriptyline, n=6 per group). Naloxone, an opioid receptor antagonist, did not alter the local effect of amitriptyline against phase 2 flinching (Figure 16B), phase 1 flinching, or biting/licking time (data not shown).

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EXAMPLE 3

The drugs tested were desipramine, fluoxetine, caffeine and propranolol (Sigma Chemical Co., St. Louis, MO), ketanserin and tropisetron (Research

25   Biochemicals Inc., Natick, NJ), and GR113808A (Glaxo Research Group, Greenford, Middlesex, U.K.). Formalin (37% formaldehyde) (British Drug Houses, Toronto, Ontario) was the control irritant. All drugs were prepared in saline and diluted to the indicated dose with formalin when coinjected.

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Rats were maintained and surgically altered by spinal nerve ligation of the 5th and 6th spinal nerves as described in Example 1. Preliminary tests showed that the spinal ligation surgery generates a thermal hyperalgesia of 2-3 sec in the paw corresponding to the ligation (baseline on non-ligated side 10-12 sec, and 710 on

5   ligated side), and this effect was maintained for at least 22 days following surgery.

A. The Formalin Test

The formalin test was applied as described in Example 2 above. Following

10   injections, rats were returned to the observation chamber and scored for flinching behaviors (lifting, shaking or rippling of the haunch) and biting/licking time. Two rats in adjacent chambers were observed at a time, with observations occurring in alternate 2 min bits. Recorded episodes were not corrected for this, so values represent about half of the total behaviors expressed.

15

B. Local Antinociceptive Action of Desipramine and Fluoxetine

When independently injected with formalin 2.5% into the dorsal hindpaw of a non-surgically altered rat, desipramine (10-300 nmol) and fluoxetine (30-300 nmol)

20   both produced a dose-dependent antinociceptive action against phase 2 flinching behaviors (Figure 17B) and phase 2 biting/licking behaviors (Figure 17C). Phase 1 flinching behaviors exhibited much more variability, and were only reduced significantly at the highest doses (Figure 17A). There was no phase 1 expression of biting/licking behaviors (Figure 18C). Desipramine action suppressed both pain

25   response behaviors (illustrated in Figures 18A-B) in a uniform manner throughout the entire time course observed. A similar pattern of suppression of behaviors was observed with fluoxetine (data not shown). However, when the doses that were most efficacious in the ipsilateral paw were injected into the contralateral hindpaw, the antinociceptive action resulting from coadministration of desipramine or fluoxetine

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with formalin was not observed. (Figures 17A-C, 18B and 18C). These results show that the antinociceptive effect of the locally injected antidepressant was local only.

C. Coadminisration of Desipramine or Fluoxetine and Caffeine

To test the effect of caffeine on the antinociceptive effect of the antidepressant drugs, a formalin concentration of 1.5% was used as this concentration revealed the involvement of adenosine in the action of amitriptyline most clearly. (See Example 2 above). Coadministration of caffeine (1500 nmol) with desipramine or fluoxetine had no significant effect on the antinociceptive actions of the antidepressants in any aspect of the formalin test (Figures 20A-C).

D. Local Administration of Desipramine and Fluoxetine in thermal hyperalgesia test

Nerve damaged rats (SNL) were anaesthetized briefly for drug injections (in 50 μl) into the dorsal part of the hindpaw, either on the side ipsilateral to the surgery (ligated side) to test for local effects, or the contralateral side to test for systemically mediated effects. Thermal thresholds were determined with the investigator unaware of the particular treatment.

The local administration of desipramine (100 nmol), but not fluoxetine (100 nmol), into the hindpaw corresponding to the operated side produced a local anti-hyperalgesic effect when reaction thresholds were determined using a thermal paw stimulator (Figures 19A and 4B). This effect was not observed following injection into the contralateral paw corresponding to the non-operated side (Figure 19B) These results show that the antinociceptive effects observed in the ipsilateral side were local. The local injection of desipramine or fluoxetine, 100 nmol each, into a normal rat paw (control) had no effect on thermal thresholds (n=4 each,

data not shown). Coadministration of caffeine (1500 nmol), which inhibits the local

action of amitriptyline on thermal hyperalgesia following spinal nerve ligation

(Example 1), also blocked the antihyperalgesic action of desipramine in the spinal

nerve ligation model (Figures 21A-C).

5

E. Effects of Desipramine, Fluoxetine and Amitriptyline on Paw Volume

Paw volume was determined by plethysmometry using a commercially

available device (Ugo, Basile). The hindpaw was inserted into the solution to the

10   junction of the hairy and non hairy skin, and volume was determined by displacement.

Triplicate baseline determinations were determined prior to the formalin injection, and

again immediately following the behavioral observation interval, which lasted 60 min.

For time course determinations, volumes were determined at 30 min intervals as

indicated up to 3 hours in separate groups of rats.

15

The anti-inflammatory effect of desipramine and fluoxetine was tested by the

independent coadministration of desipramine and fluoxetine (doses of 100 or

300 nmol) with formalin. No significant effect on the increase in paw volume induced

by 1.5% formalin alone was observed by the antidepressants, although there was a

20   tendency towards an increased paw volume resulting from fluoxetine (Figure 22A) at

2.5% formalin. When administered into the contralateral hindpaw, fluoxetine also

significantly increased the paw volume in the absence of an inflammatory stimulus

(Figure 22B). The time course of the actions of desipramine and fluoxetine was also

determined. In these tests desipramine produced a limited effect on paw volume

25   (Figure 23A), but fluoxetine produced a clear dose-related increase in paw volume

that was maintained for the entire 3 hr observation interval (Figure 23B). The

increase in paw volume was not accompanied by a reddening of the paw, or by

spontaneous behaviors indicative of discomfort (e.g. lifting, licking, shaking).

Amitriptyline also produced a limited increase on paw volume (Figure 23C), and did

46

not significantly alter the degree of paw swelling produced by formalin 1.5% (data not shown).

F. Effects of Receptor Subtypes in Nociceptive Action of Desipramine and
5 **Fluoxetine**

To test for involvement of 5-HT receptor subtypes in the action of fluoxetine (100 nmol), the effects of propranolol (5-HT$_1$ receptor antagonist) (300 nmol), ketanserin (5-HT$_2$ receptor antagonist) (500 nmol), tropisetron (5-HT$_{3/4}$ receptor
10 antagonist) (300 nmol), and GR113808A (5-HT$_4$ receptor antagonist) (500 nmol) on paw volume were determined at doses known to block 5-HT actions when injected into the hindpaw (G.J. Doak and J. Sawynok (1997) Neuroscience 80:939-949). The results of these tests show that the increase in paw volume induced by fluoxetine was blocked by ketanserin but not by any of the other antagonists, indicating an
15 involvement of 5-HT$_2$ receptor subtypes in this action (Figures 24A-B).

EXAMPLE 4

A.   The Role of 5-HT$_2$ Receptor Antagonism in Peripheral Analgesia by
20 **Amitriptyline**

Studies were conducted to resolve an apparent inconsistency in the art regarding the mechanism of analgesia of amitriptyline and other tricyclic, second generation, or third generation antidepressants of the invention. H. Hall et al. first
25 suggested that a number of antidepressants can block 5-HT$_2$ receptors ((1984) *Acta Pharmacol Toxicol,* 54:379-384). More recently F.V. Abott et al. demonstrated that peripheral 5-HT$_2$ receptor blockers can block the expression of formalin-evoked behaviors using the formalin test as described above, except that 1% formalin was used and proposed based on the results of this low dose study that 5-HT$_2$ receptor
30 antagonists might make useful analgesics ((1996) *Neuropharmacology* 35:99-110).

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However, when Doak and Sawynok, supra (1997) conducted a detailed investigation into the 5-HT receptor subtypes involved in formalin-evoked behaviors using a 2.5% formalin test, evidence was obtained for the involvement of 5-HT_1, 5-HT_3 and 5-HT_4 but not 5-HT_2 receptors in such behaviors. Figure 25 above summarizes the results of the Doak and Sawynok studies of 1997.

A number of drug effects are known to be intensity-dependent (in their expression) in the formalin test (e.g., Sawynok J., Reid AR (1996) *Eur J Pharmacol,* 298:105-111). In view of the apparent contradiction between the results obtained in the Abott study in which 1% formalin was used and in the Doak and Sawynok study, in which 2.5% formalin was used, studies were undertaken to re-evaluate the performance of ketanserin, the selective 5-HT_2 receptor antagonist used in both studies.

As the Abbott study used 1% formalin rather than the 2.5% formalin used in the Doak and Sawynok study, the lower dosage of formalin was used to re-evaluate the performance of ketanserin using individual groups of male Sprague Dawley rats (n=6 per group) to conduct the 1% formalin test as in Example 2 A. above. The results of the re-evaluation shown in Figure 26 above indicate ketanserin at doses of 50 nmol and 500 nmol (a dose equal to or greater than the 9.5-50 µg or 0.92 - 92 nmol used in the Abott study) fails to block formalin-evoked flinches in Sprague Dawley rats even at 1% formalin. By contrast, co-administration of 3 nmol and 30 nmol doses of ketanserin were used to blockade change in paw volume induced by local injection of 5-HT into the hindpaw of Sprague Dawley rats. Drugs were injected in a volume of 50 µl and paw edema was measured by plethysmometry (volume displacement). The results of these studies shown in Figures 27 A and B clearly indicate that the dosage of ketanserin that failed to prevent 1% formalin-induced flinching behavior was sufficient to block the paw edema produced by local injection of 5-HT.

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The only other difference between the Abott study and the Doak and Sawynok study was the species of rat used, Long Evans rats in the former and Sprague Dawley rats in the latter. It may be that there is a differential expression of 5-HT receptors on sensory afferent nerve terminals in the two species. However, it should be noted that

5   in the absence of any effect of the 5-HT$_2$ antagonist in the test in species and conditions where a profound analgesia is seen with amitriptyline (2.5% formalin, Sprague Dawley rats), it is not shown that 5-HT$_2$ antagonism accounts for the analgesia demonstrated by amtitriptyline and other tricyclic, second generation, or third generation antidepressants observed in the studies described herein.

10

B.   The Role of Blockade of Cholinergic Muscarinic and Nicotinic Receptors in Peripheral Analgesia by Amitriptyline

Amitriptyline is known to bind to central muscarinic cholinergic receptors

15   (Hall H, Ogren SO. (1981) *Eur J. Pharmacol*, 70:393-407; Choi A, Mitchelson F. (1994) *Pharmacology*, 48:293-300), and its affinity for such receptors has been implicated in certain side effects of amitriptyline observed clinically (Baldessarini R.J. (1990) *The Pharmacological Basis of Therapeutics*, 383-435). Amitriptyline also blocks nicotinic receptor-mediated responses (Schofiled G.G. et al. (1981) *Proc Natl*

20   *Acad Sci USA*, 78:5420-5244; Fryer J.D., Lukas R.J. (1999) *J Neurochem*, 72:1117-1124), although in this case the block seems to result from an inhibition of the ion channel itself rather than a competitive inhibition at the receptor binding site. Acetylcholine can stimulate sensory nerve endings to produce pain (Steen K.H., Reeh P.W. (1993) *J Neurophysiol*, 70:397-405; Bauer M.B. et al. (1994) *Neuroscience*,

25   62:351-359), and both nicotinic and muscarinic receptors are present in dorsal root ganglion neurons and on sensory nerve endings (Ninkovic M., Hunt S.P. (1983) *Brain Research*, 272:57-69; Wamsley J.K. et al. 1981) *Brain Research*, 217:155-161). In view of these findings, studies were conducted to determine whether established

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antagonists for muscarinic and nicotinic cholinergic receptors can mimic the action of
amitriptyline.

In these studies, groups of male Sprague Dawley rats (n=5-8 per group)
received doses ranging from 10 to 1000 nmol of atropine (muscarinic receptor
antagonist), mecamylamine (ganglionic nicotinic receptor antagonist) and
d-tubocurarine (neuromuscular nicotinic receptor antagonist) coadministered with
formalin in the formalin 2.5% test. Figures 28A-C show the effects of the drugs on
phase 1 (Figure 28A) and phase 2 flinching (Figure 28B) and phase 2 biting/licking
behaviors (Figure 28C), which were determined as described above.

In these tests, atropine produced a reduction in flinching behaviors only at the
highest dose tested (1000 nmol). This effect is a local peripheral action, as it was not
observed following administration of atropine into the contralateral hindpaw.
Atropine also produced a dose-dependent reduction in biting/licking behaviors, and
this appeared to be a composite of peripheral and central actions as administration of
atropine into the contralateral hindpaw also produced a reduction in this behavior at
the highest dose (Figure 28C). Mecamylamine had no significant effect on either
behavior at the highest dose. d-Tubocurarine had no effect on formalin-evoked
behaviors at 10 nmol, and at 100 nmol, d-tubocurarine was lethal, probably due to
systemic activity of the drug on respiratory muscles causing respiratory arrest.

These results indicate that established cholinergic receptor antagonists are only
weakly active (atropine) or are inactive (mecamylamine, d-tubocurarine) at inhibiting
formalin-evoked behaviors when administered peripherally. In view of these results,
it can be concluded that the actions of amitriptyline on formalin behaviors are due to
blockade of peripheral cholinergic receptors on sensory nerve terminals.

50

C.   Conclusions

The present results provide no evidence to support an involvement of blockade of peripheral 5-HT$_2$, muscarinic cholinergic or nicotinic cholinergic receptors in the

5   analgesic actions of amitriptyline in the rat formalin test. To date, despite considering the possibility of a potential involvement of antihistaminic, antiadrenergic, antiserotonergic and anticholinergic actions in the action of amitriptyline, the only mechanism clearly implicated in the action of amitriptyline is some form of interaction with adenosine receptors as peripheral analgesia is partially blocked by the

10   adenosine receptor antagonist caffeine.

It will be apparent to those skilled in the art that various changes may be made in the invention without departing from the spirit and scope thereof, and therefore, the invention encompasses embodiments in addition to those specifically disclosed in the

15   specification, but only as indicated in the appended claims.

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That which is claimed is:

1.   A method for producing local analgesia in a subject having a site of local
discomfort, said method comprising locally administering an effective amount of a
tricyclic, second generation or third generation antidepressant to the site.

2.   The method according to claim 1 wherein the antidepressant is a tricyclic
antidepressant.

3.   The method according to claim 1 wherein the tricyclic antidepressant has a
structure:

$$Ar_1-Z(R)-Ar_2$$

        wherein Z is a 7-membered ring, optionally containing 1 or 2 biocompatible
5   heteroatoms, or an 8-membered bicyclic ring,

        Ar_1 and Ar_2 are optionally substituted aromatic rings fused to Z, and

        R is an alkylamino or arylamino substituent, optionally an N-oxide derivative
thereof.

4.   The method according to claim 3 wherein the heteroatom is oxygen or
nitrogen.

5.   The method according to claim 3 wherein Ar_1 and Ar_2 are independently
optionally substituted with a biocompatible halogen.

6.   The method according to claim 3 wherein the alkylamino or arylamino
substituent is an N-oxide derivative thereof.

7.   The method according to claim 3 wherein the alkylamino substituent comprises from 4 to 5 carbons atoms.

8.   The method according to claim 7 wherein the alkylamino is a tertiary or secondary amino group.

9.   The method according to claim 3 wherein R is selected from the group consisting of

$-(CH_2)_3N(CH_3)_2$,

$-(CH_2)_3NHCH_3$,

$-(CH_2)_3N(CH_3)_2$,

$-CH_2CH(CH_3)CH_2N(CH_3)_2$,

$=CH(CH_2)_2N(CH_3)_2$,

$-(CH_2)_3NHCH_3$, and

$=CH(CH_2)_2NHCH_3$.

10.   The method according to claim 1 wherein the tricyclic antidepressant is selected from the group consisting of clomipramine, imipramine, amitryptyline, doxepin, desipramine, nortriptyline, amoxapine, maprotiline, trimipramine, and suitable combinations of any two or more thereof.

11.   The method according to claim 1 wherein the tricyclic antidepressant is amitriptyline or desipramine.
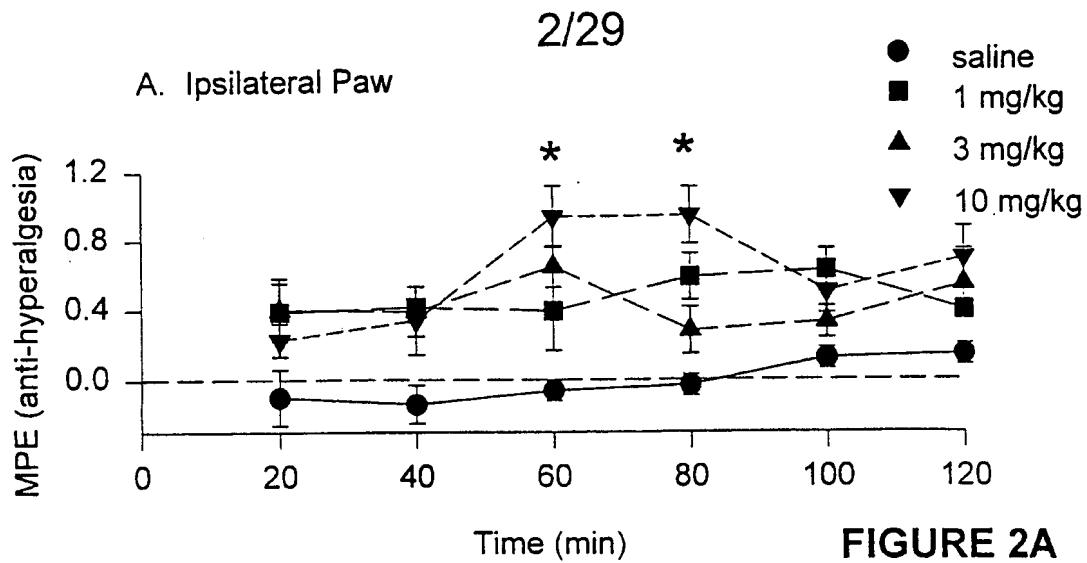
12.   The method according to claim 1 wherein the second generation or third generation antidepressant is selected from the group consisting of mirtazapine, venlafaxine, trazodone, bupropion, fefazodone, and suitable combinations of any two or more thereof.

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13.   The method according to claim 12 wherein the second generation or third generation has the structure
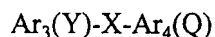
$$Ar_3(Y)\text{-}X\text{-}Ar_4(Q)$$

wherein Ar_3 is an N-containing heterocyclic ring,

Y is either an aryl group fused to the heterocyclic ring or one or two substituents selected from alkyl, arylalkyl, arylalkyloxy, aryl and heteroaryl substituents comprising a total of about 4 to 8 carbons attached to Ar_3,

and attached to Q at a second nitrogen atom of Ar_4.

14.   The method according to claim 13 wherein the X is an alkyl group containing 3 carbons.

15.   The method according to claim 13 wherein Ar_3 is a 1,2,4-triazone, Q is an heteroarylalkyl substituent containing 6 to 8 carbon atoms, and is substituted at the 4 position with Q.

16.   The method according to claim 13 wherein the alkyl substituent contains 3 carbon atoms.

17.   The method according to claim 13 wherein the benzene ring is substituted with a halogen selected from the group consisting of chlorine, bromine, and fluorine.

18.   The method according to claim 1 wherein the tricyclic-antidepressant is desipramine.

19.   The method according to claim 1 wherein the tricyclic-antidepressant is amitriptyline.

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20.   The method according to claim 1 wherein the tricyclic-antidepressant is administered topically.

21.   The method according to claim 1 wherein the antidepressant is administered as a formulation selected from the group consisting of a cream, a lotion, a gel, an ointment, a spray, a polymer stabilized crystal, a powder, and an aerosol.

22.   The method according to claim 17 wherein the antidepressant is administered by a method selected from injection, supersonic powder injection, and transdermal electroporation.

23.   The method according to claim 1 wherein the antidepressant is administered encapsulated in a delivery system selected from the group consisting of a microsphere, a polymer stabilized crystal, and a liposome.

24.   The method according to claim 1 wherein the analgesia suppresses pain caused by inflammation.

25.   The method according to claim 1 wherein the analgesia suppresses neuropathic pain.

26.   A composition for local administration comprising a tricyclic or heterocyclic antidepressant other than doxepine, and a vehicle suitable for topical administration.

27.   The composition according to claim 26 wherein the antidepressant is a tricyclic antidepressant.

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28.   The composition according to claim 26 wherein the tricyclic antidepressant has a structure:

$$Ar_1-Z(R)-Ar_2$$
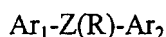
       wherein Z is a 7-membered ring, optionally containing 1 or 2 biocompatible
5   heteroatoms, or an 8-membered bicyclic ring,

       Ar_1 and Ar_2 are optionally substituted aromatic rings fused to Z, and

       R is an alkyl amino or aryl amino substituent, and optionally an N-oxide derivative thereof.

29.   The composition according to claim 28 wherein the heteroatom is oxygen or nitrogen.

30.   The composition according to claim 28 wherein Ar_1 and Ar_2 are independently optionally substituted with a biocompatible halogen.

31.   The composition according to claim 28 herein the alkylamino or arylamino substituent is an N-oxide derivative thereof.

32.   The composition according to claim 28 wherein the alkylamino substituent comprises from 4 to 5 carbons atoms.

33.   The composition according to claim 32 wherein the alkylamino is a tertiary or secondary amino group.

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34.   The composition according to claim 28 wherein R is selected from the group
consisting of

$-(CH_2)_3N(CH_3)_2$,

$-(CH_2)_3NHCH_3$,

$-(CH_2)_3N(CH_3)_2$,

$-CH_2CH(CH_3)CH_2N(CH_3)_2$,

$=CH(CH_2)_2N(CH_3)_2$,

$-(CH_2)_3NHCH_3$, and

$=CH(CH_2)_2NHCH_3$.

35.   The composition according to claim 26 wherein the tricyclic antidepressant is
selected from the group consisting of clomipramine, imipramine, amitryptyline,
desipramine, nortriptyline, amoxapine, maprotiline, trimipramine, and suitable
combinations of any two or more thereof.

36.   The composition according to claim 26 wherein the tricyclic antidepressant is
amitriptyline or desipramine.

37. The composition according to claim 26 wherein the second or third generation antidepressant has a structure:

$$Ar_3(Y)-X-Ar_4(Q)$$
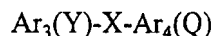
wherein Ar_3 is a substituted N-containing heterocyclic ring,

5   Y is either an aryl group fused to the heterocyclic ring, or one or two substituents selected from the group consisting of alkyl, alkyloxy, arylalkyl, arylalkyloxy, aryl, heteroaryl substitutents, and combinations thereof comprising a total of about 4 to 8 carbons attached to Ar_3,

X is an alkyl group comprising 2 to 5 carbon atoms linking Ar_3 and Ar_4,

10   Ar_4 is a piperazine attached to X by a first nitrogen atom of Ar_4, and

Q is a benzene ring optionally substituted with a biocompatible halogen and attached to Ar_4 at a seond nitrogen atom of Ar_4.

38. The composition according to claim 37 wherein the X is an alkyl group containing 3 carbons.

39. The composition according to claim 37 wherein Ar_3 is a 1,2,4-triazone substituted at the 4 position with the arylalkyoxy substituent containing 6 to 8 carbon atoms.

40. The composition according to claim 39 wherein the heteroarylalkyl substituent contains an oxygen atom.

41. The composition according to claim 37 wherein the benzene ring is substituted with a halogen selected from the group consisting of chlorine, bromine, and fluorine.

42. The composition of claim 23 further comprising an inert carrier.

43. The composition of claim 42 wherein the inert carrier is selected from the group consisting of water, isopropyl alcohol, gaseous fluorocarbons, ethyl alcohol, polyvinyl pyrrolidone, propylene glycol, a fragrance, a gel-producing material, stearyl alcohol, stearic acid, spermaceti, sorbitan monooleate, methylcellulose, and suitable combinations thereof.

44. The composition according to claim 26 wherein the composition further comprises a penetration enhancing agent.

45. The composition according to claim 26 wherein the tricyclic-antidepressant is selected from the group consisting of desipramine, amitriptyline and a suitable combination thereof.

46. The composition according to claim 26 wherein the composition further comprises a penetration enhancing agent.

47. The composition according to claim 26 wherein the tricyclic-antidepressant is desipramine.

48. The composition according to claim 26 wherein the tricyclic-antidepressant is amitriptyline.

49. The composition according to claim 26 in a formulation selected from the group consisting of a cream, a lotion, a gel, an ointment, a spray, a powder, a polymer stabilized crystal, and an aerosol.

50. The composition of claim 26 further comprising a neutralizing agent.

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51.   The composition of claim 26 wherein the composition is formulated for local injection.

52.   The composition according to claim 26 wherein the antidepressant is encapsulated in a slow release delivery vehicle.

53.   The composition according to claim 52 wherein the delivery vehicle is selected from a liposome, a microcapsule, a polymer stabilized crystal.

FIGURE 1

A. Ipsilateral Paw

FIGURE 2A

B. Contralateral Paw

FIGURE 2B

C. Cumulative MPE (ΣMPE)

FIGURE 2C

A. Ipsilateral Paw

FIGURE 3A

B. Contralateral Paw

FIGURE 3B

C. Cumulative MPE (ΣMPE)

FIGURE 3C

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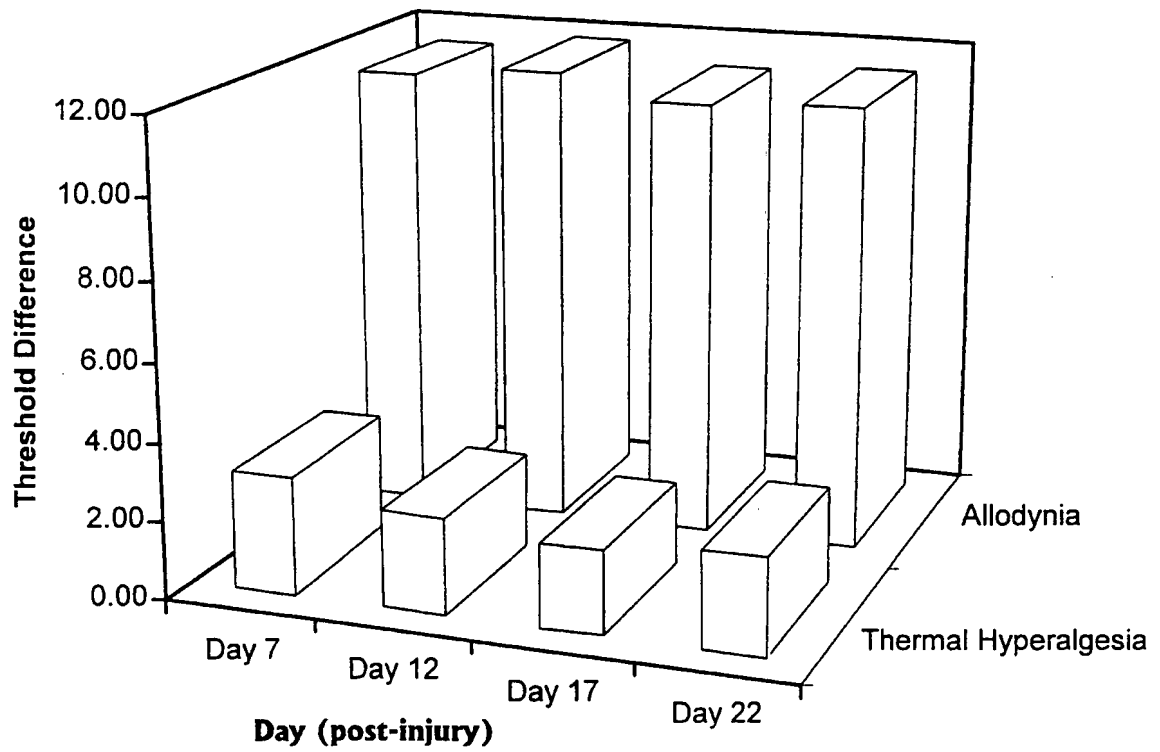
A. Ipsilateral Paw
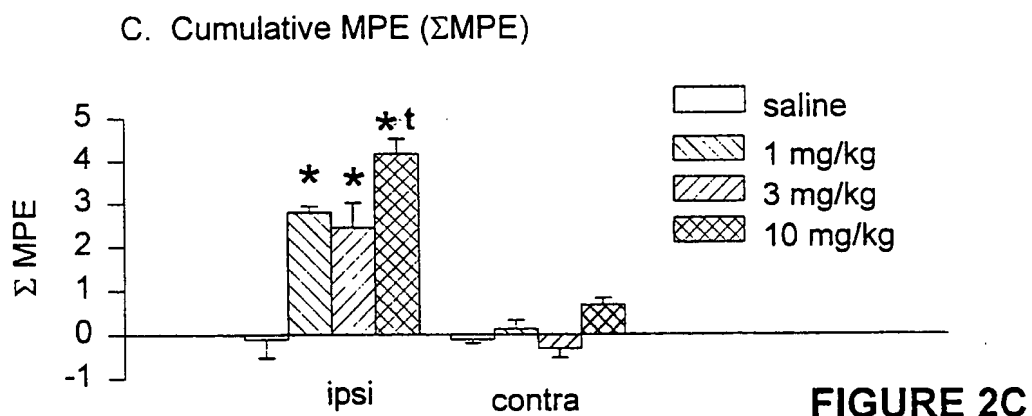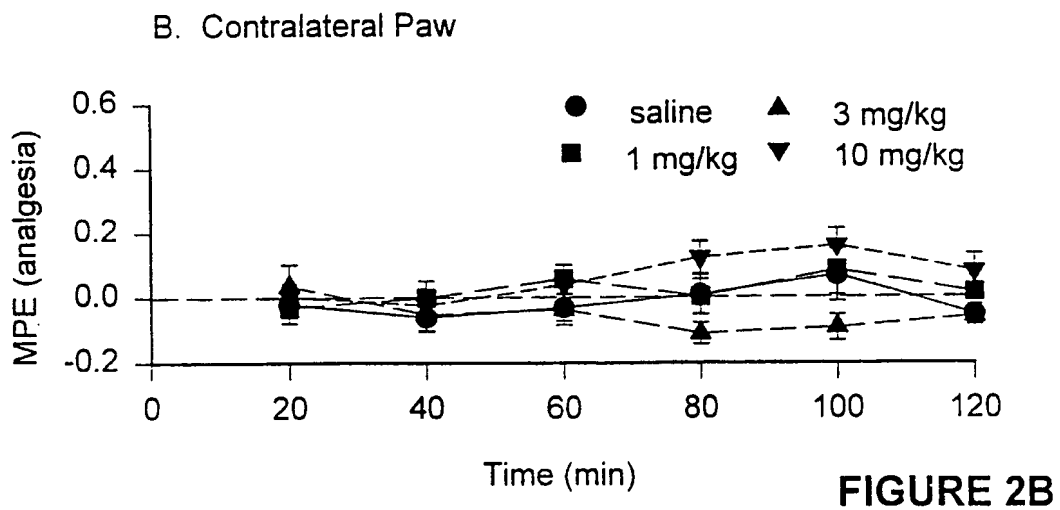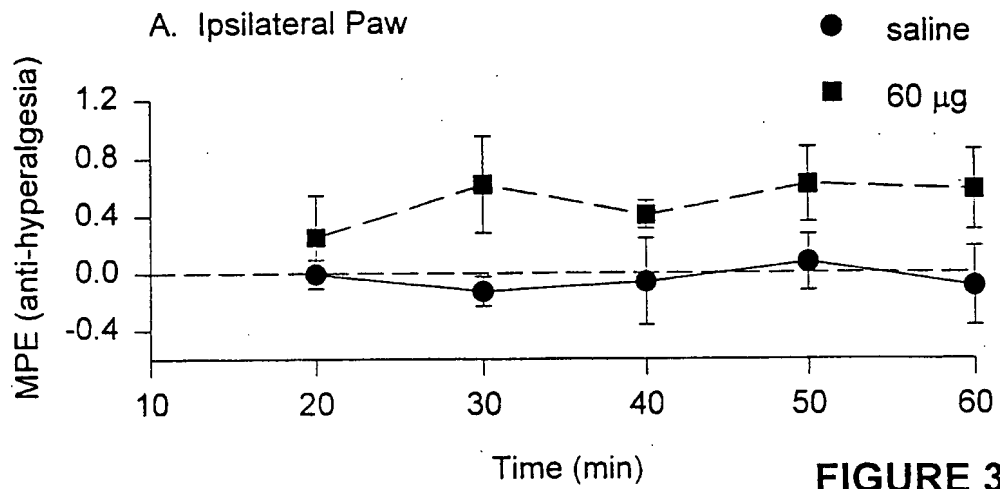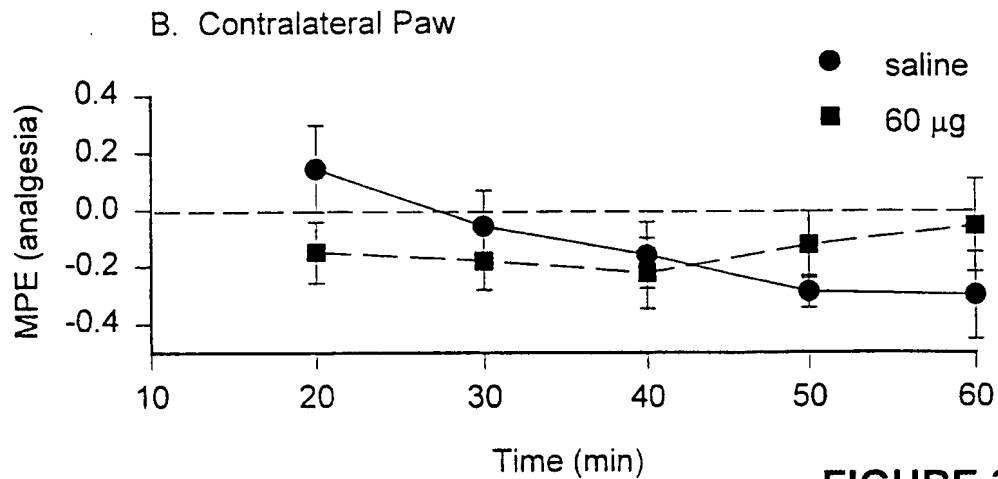
FIGURE 4A
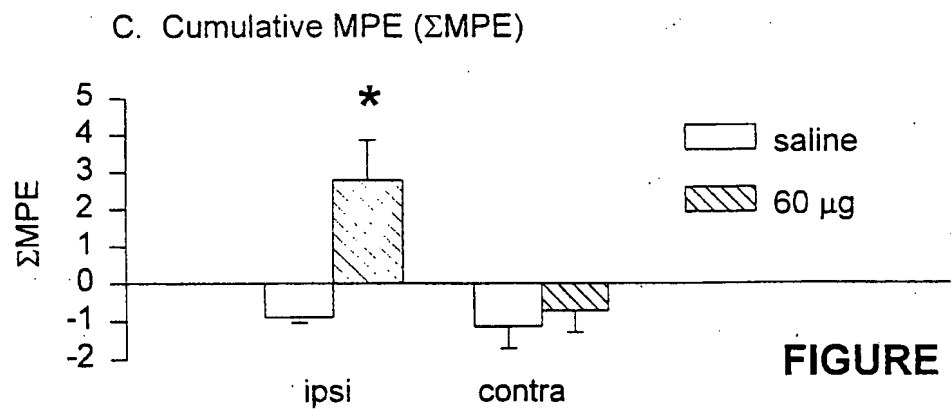
B. Contralateral Paw
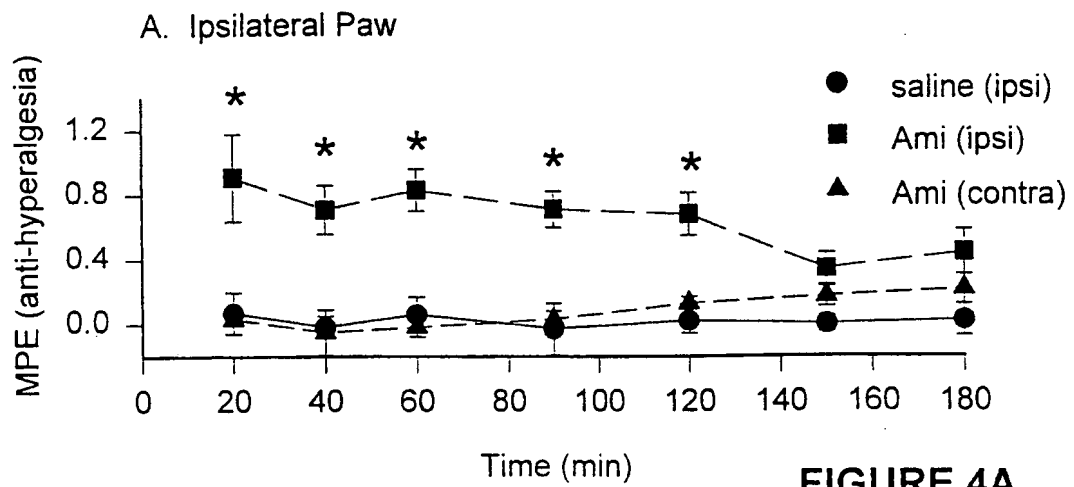
FIGURE 4B
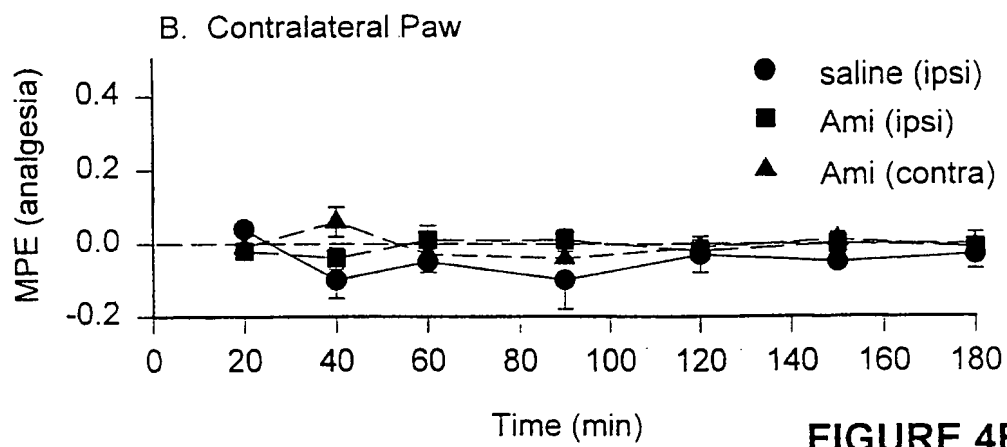
C. Cumulative MPE (ΣMPE)
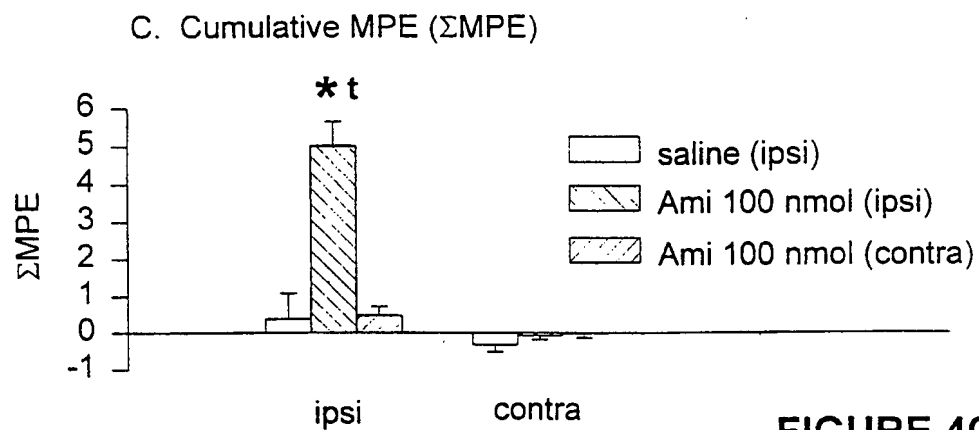
FIGURE 4C

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A. Ipsilateral Paw
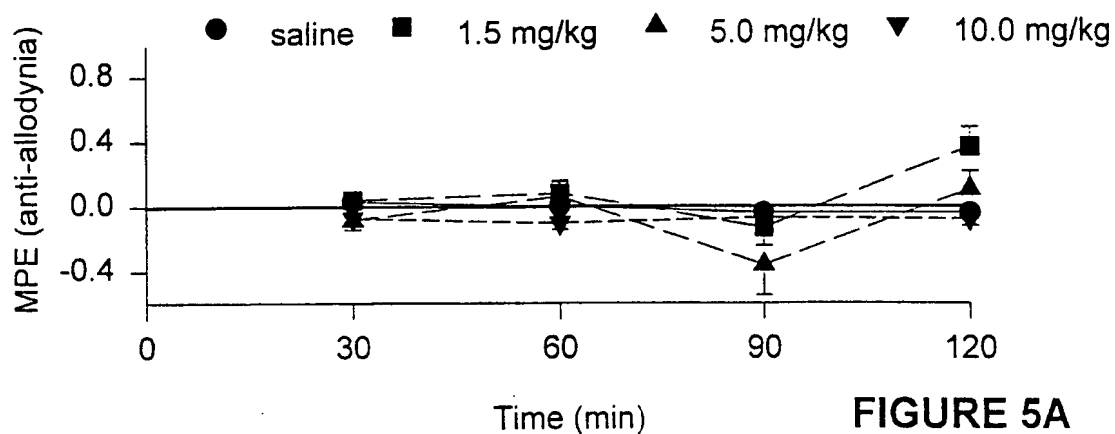
FIGURE 5A

B. Contralateral Paw
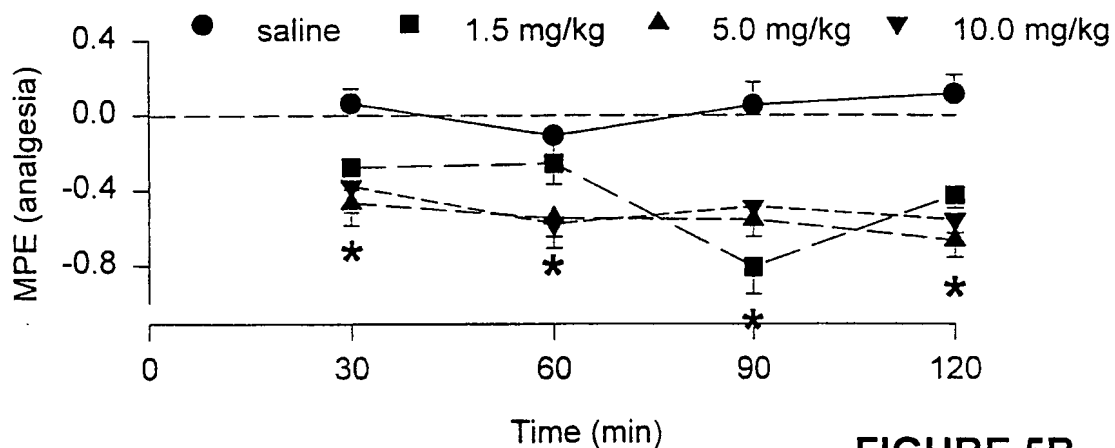
FIGURE 5B

C. Cumulative MPE (ΣMPE)

FIGURE 5C

A. Ipsilateral Paw

● saline ■ 30μg ▲ 60μg ▼ 90μg

FIG. 6A

B. Contralateral Paw

● saline ■ 30 μg ▲ 60 μg ▼ 90 μg

FIG. 6B

C. Cumulative change in MPE (ΔMPE)
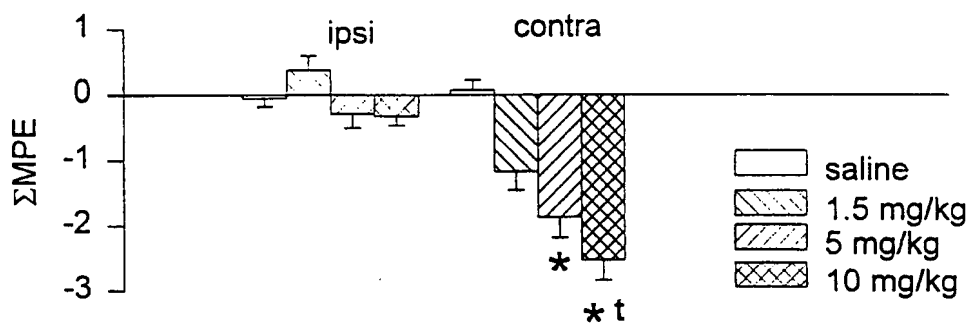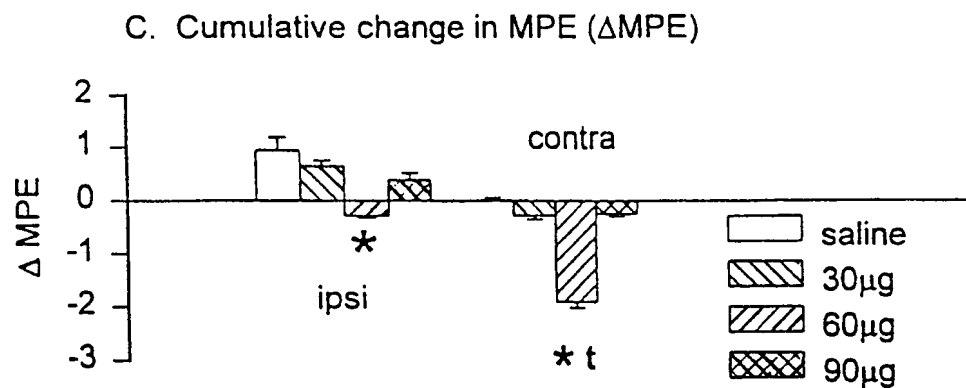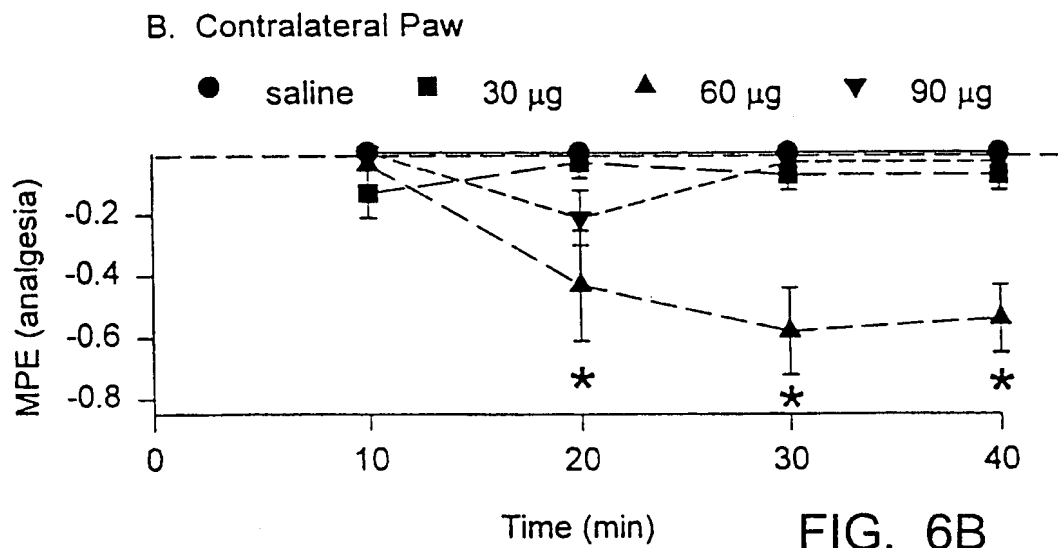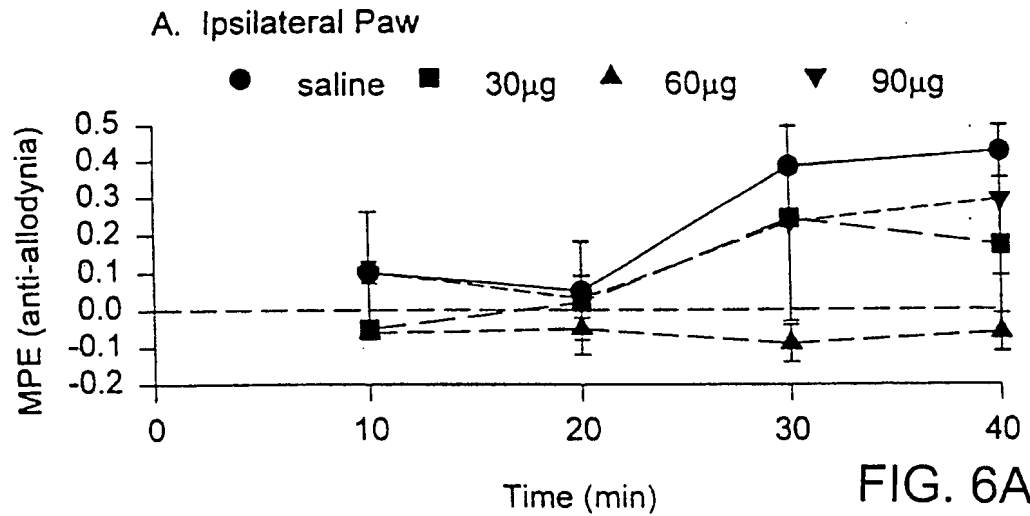
FIG. 6C

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A. Ipsilateral Paw
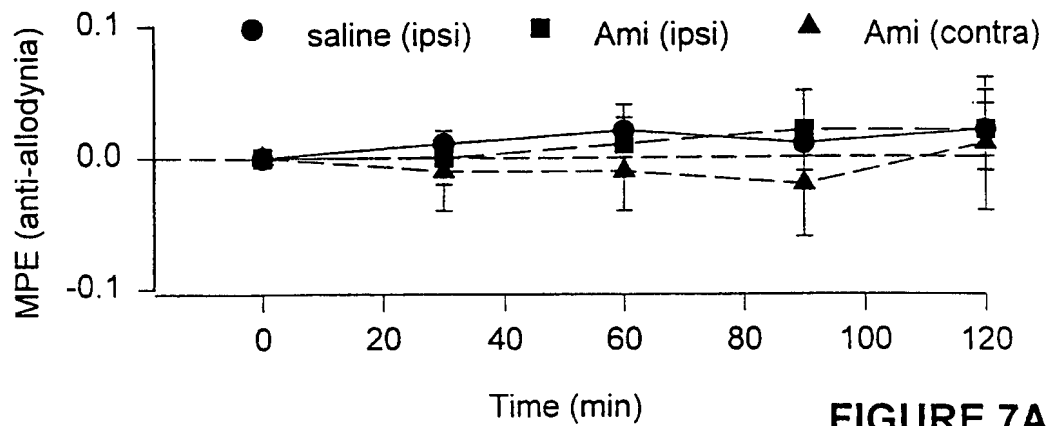
FIGURE 7A

B. Contralateral Paw
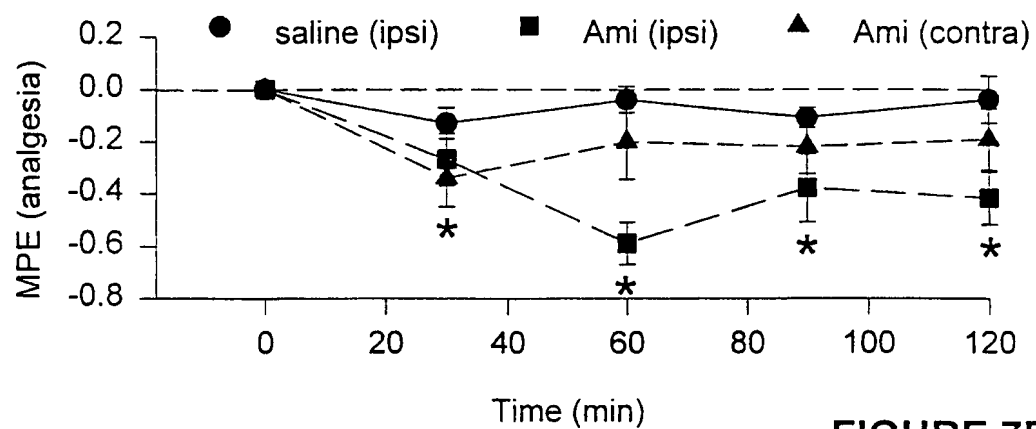
FIGURE 7B

C. Cumulative MPE (ΣMPE)
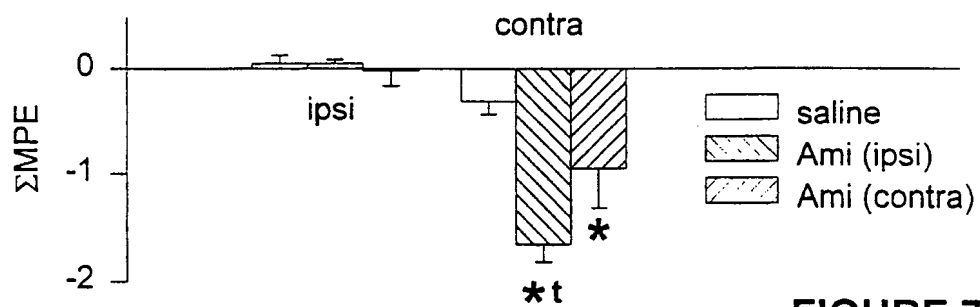
FIGURE 7C

FIGURE 8B

FIGURE 8A
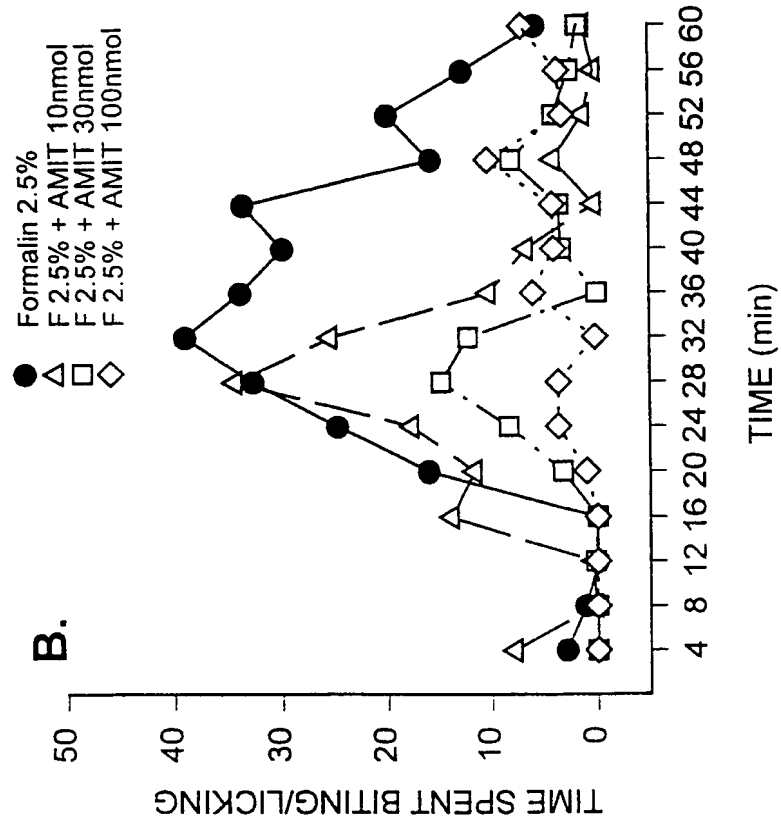
FIGURE 9A
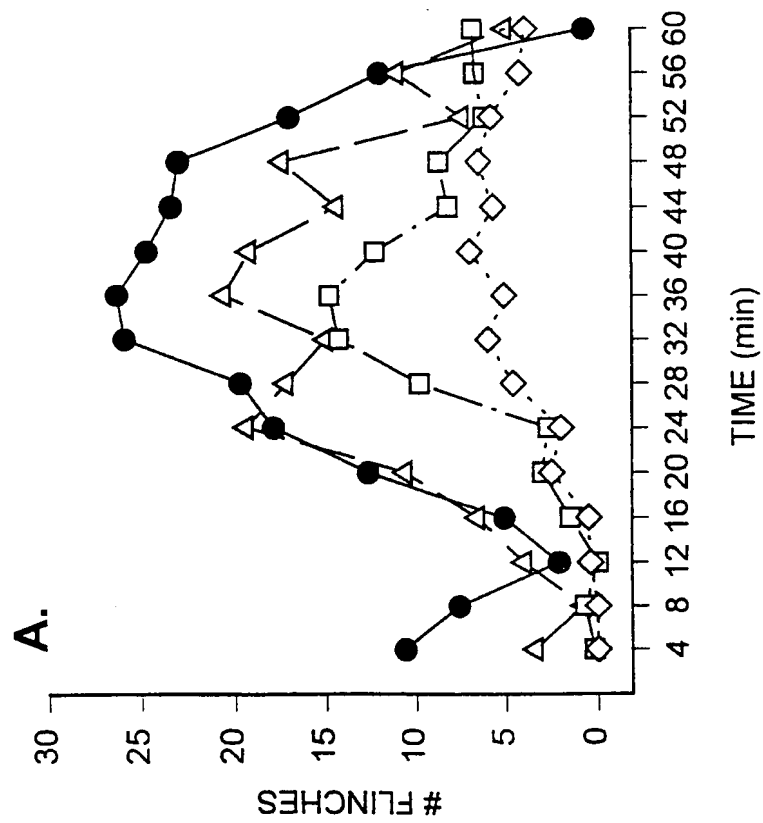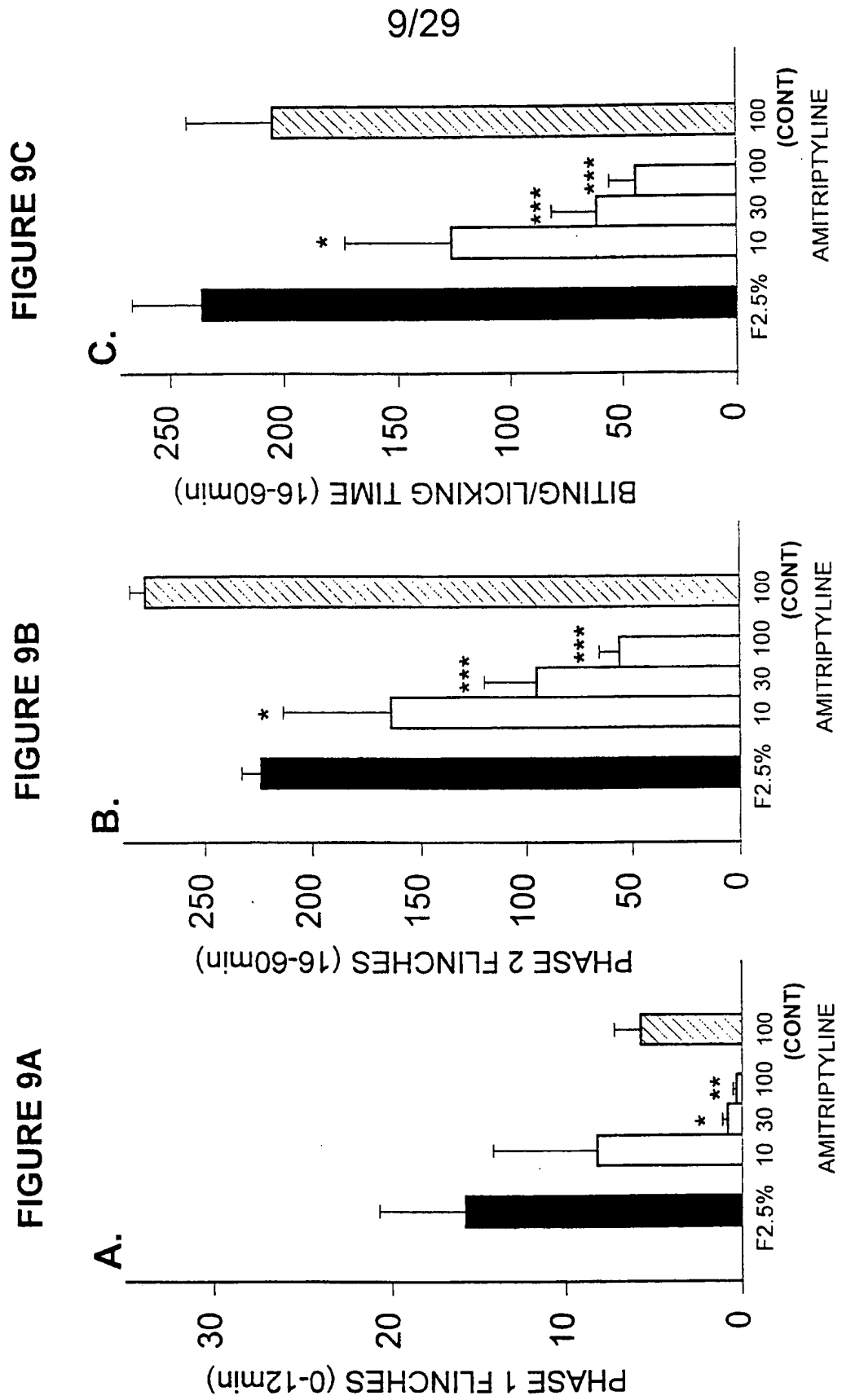
FIGURE 9B

FIGURE 9C

FIGURE 10A

FIGURE 10B

FIGURE 10C

FORMALIN CONCENTRATION
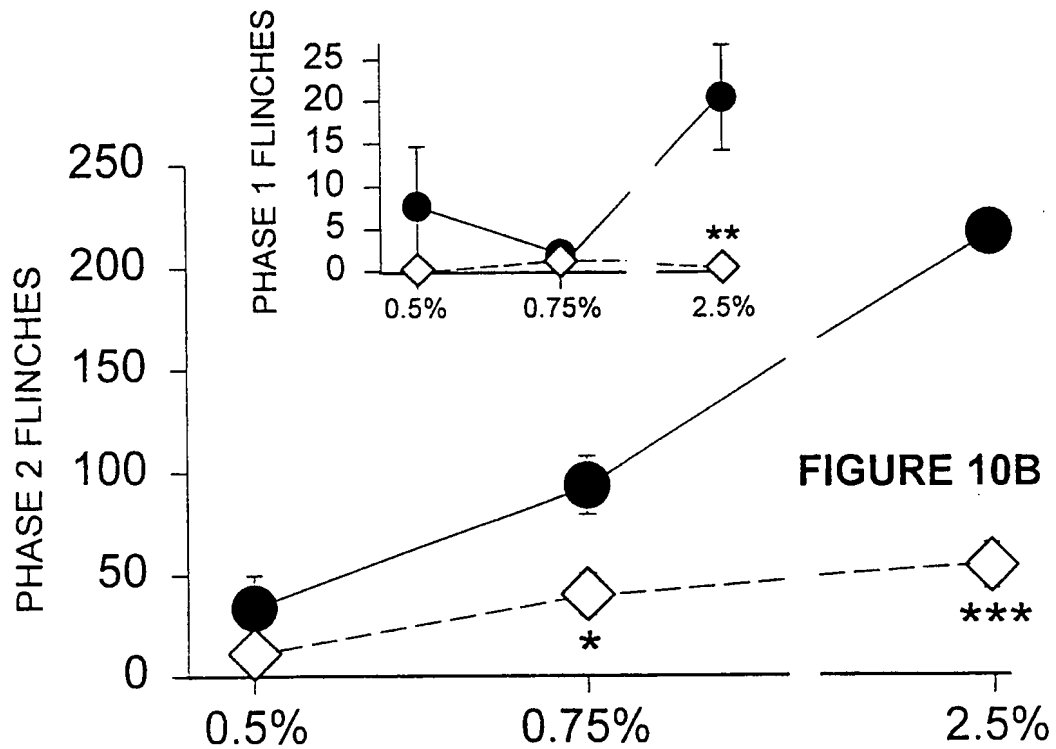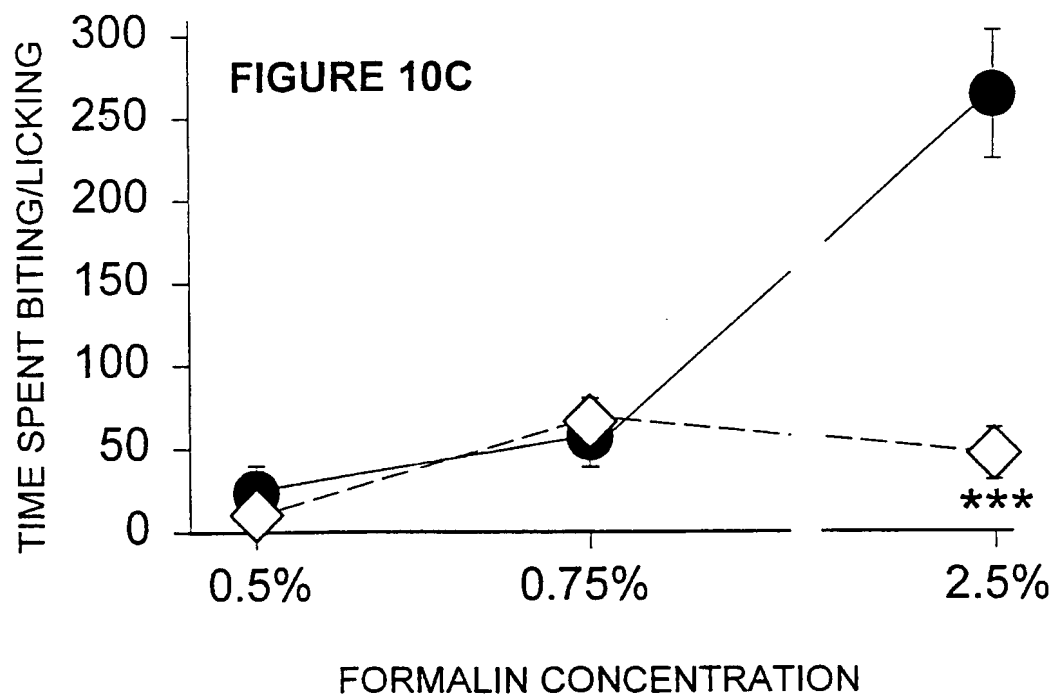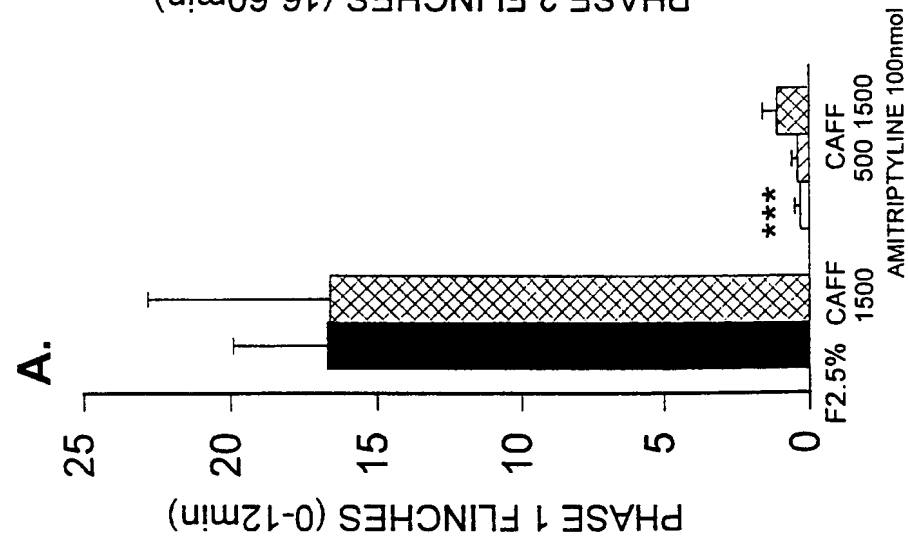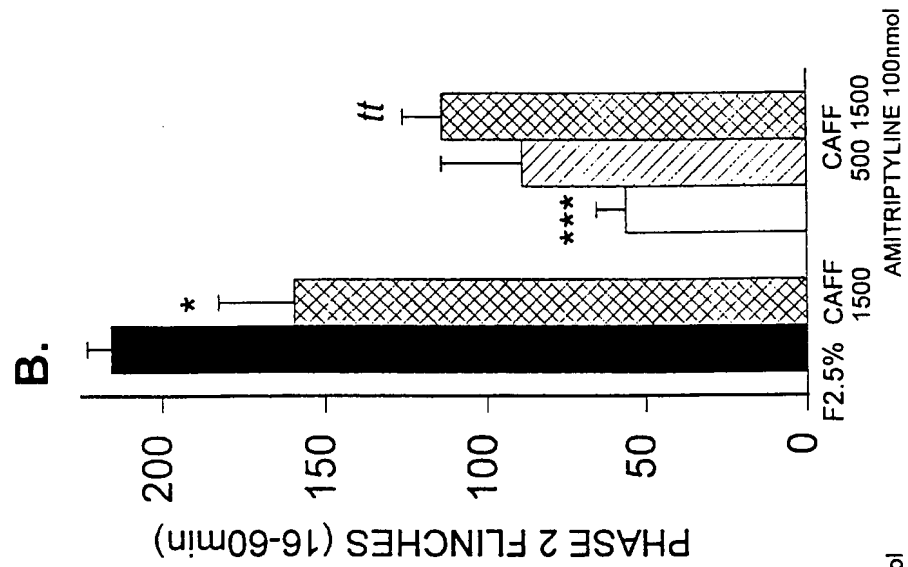
FIGURE 11A

FIGURE 11B

FIGURE 11C

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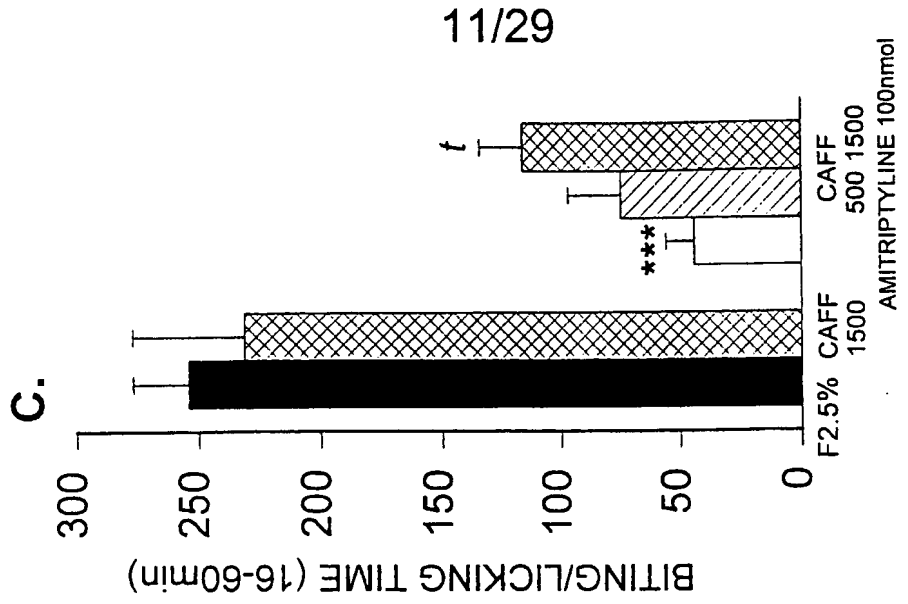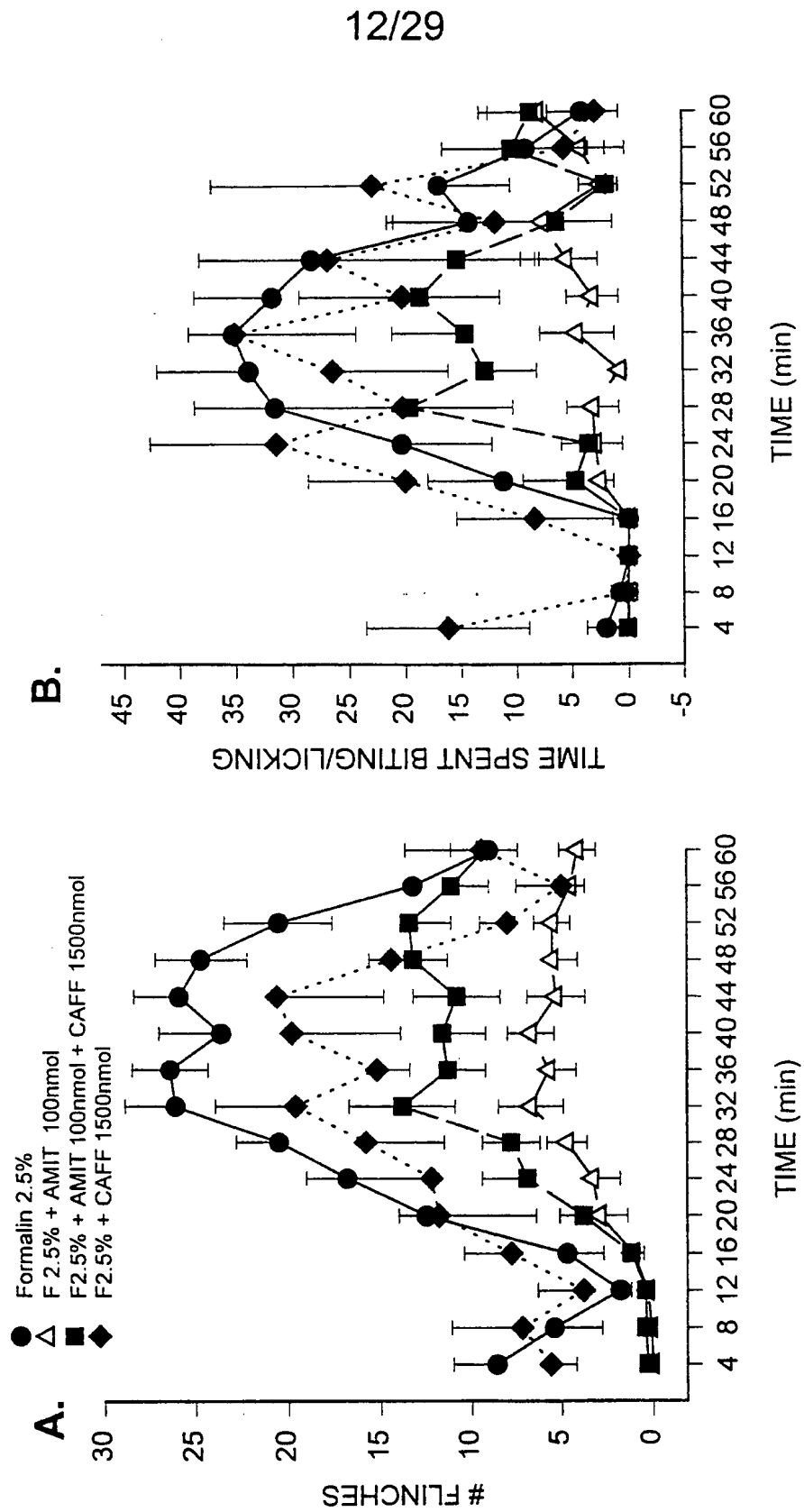
FIGURE 12B

FIGURE 12A
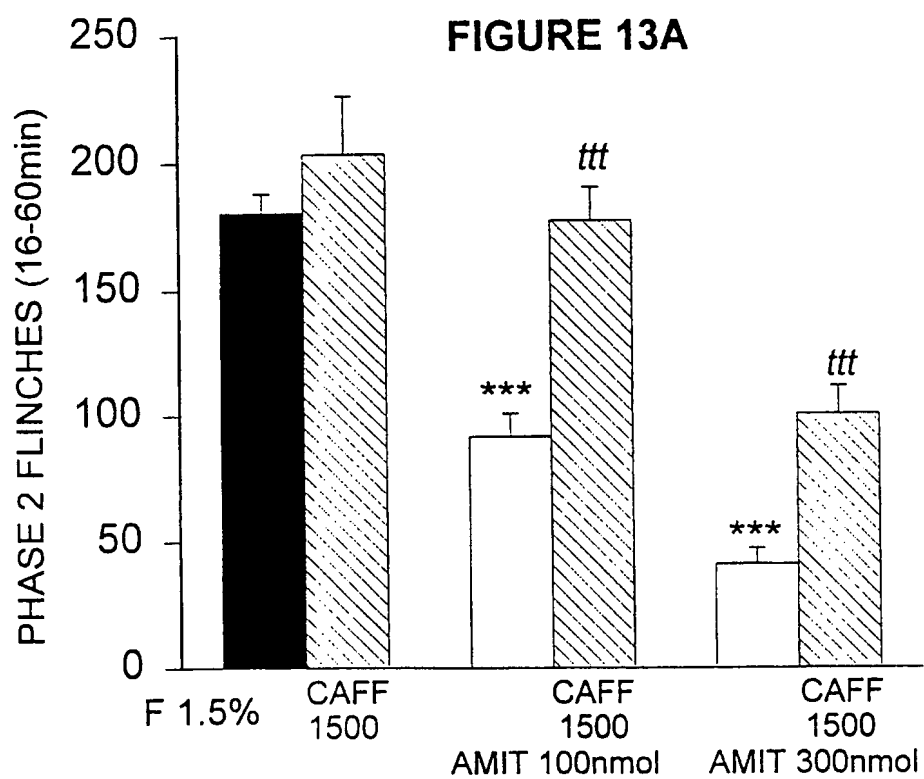
FIGURE 13A

FIGURE 13B
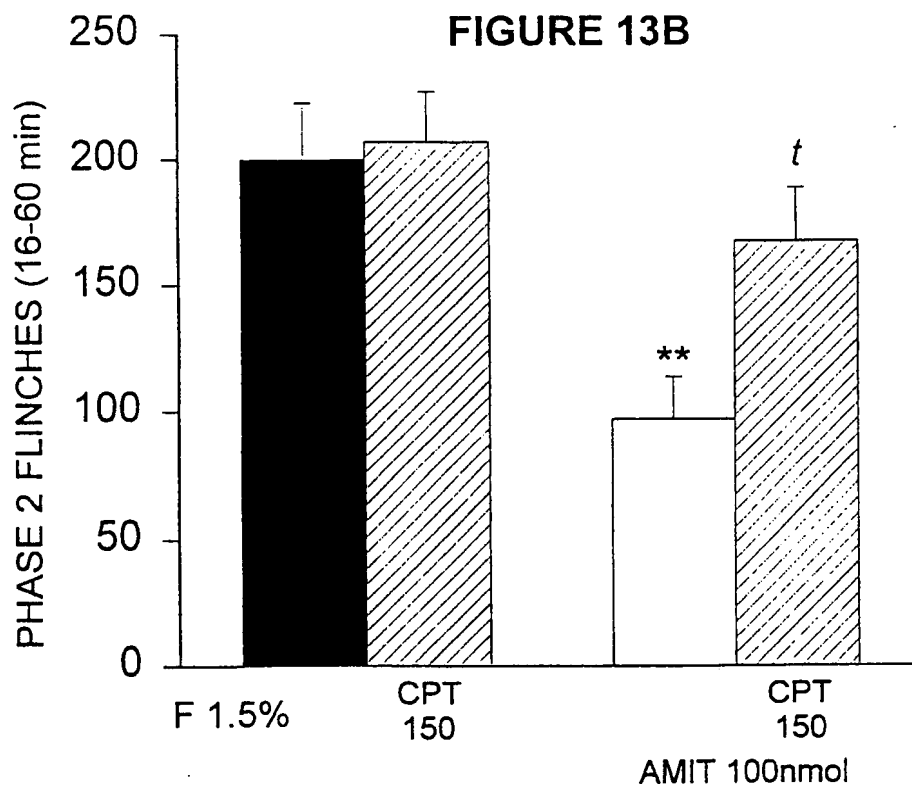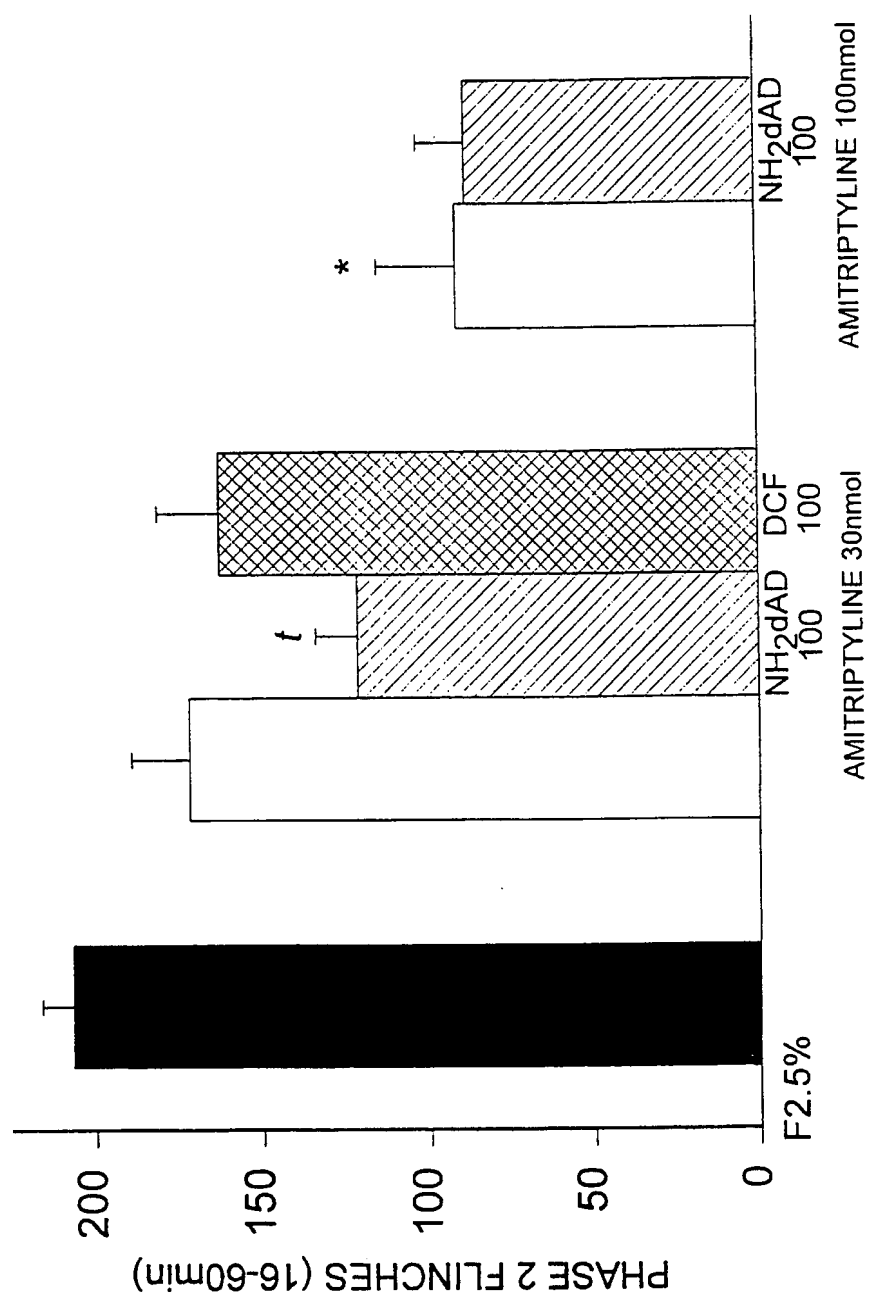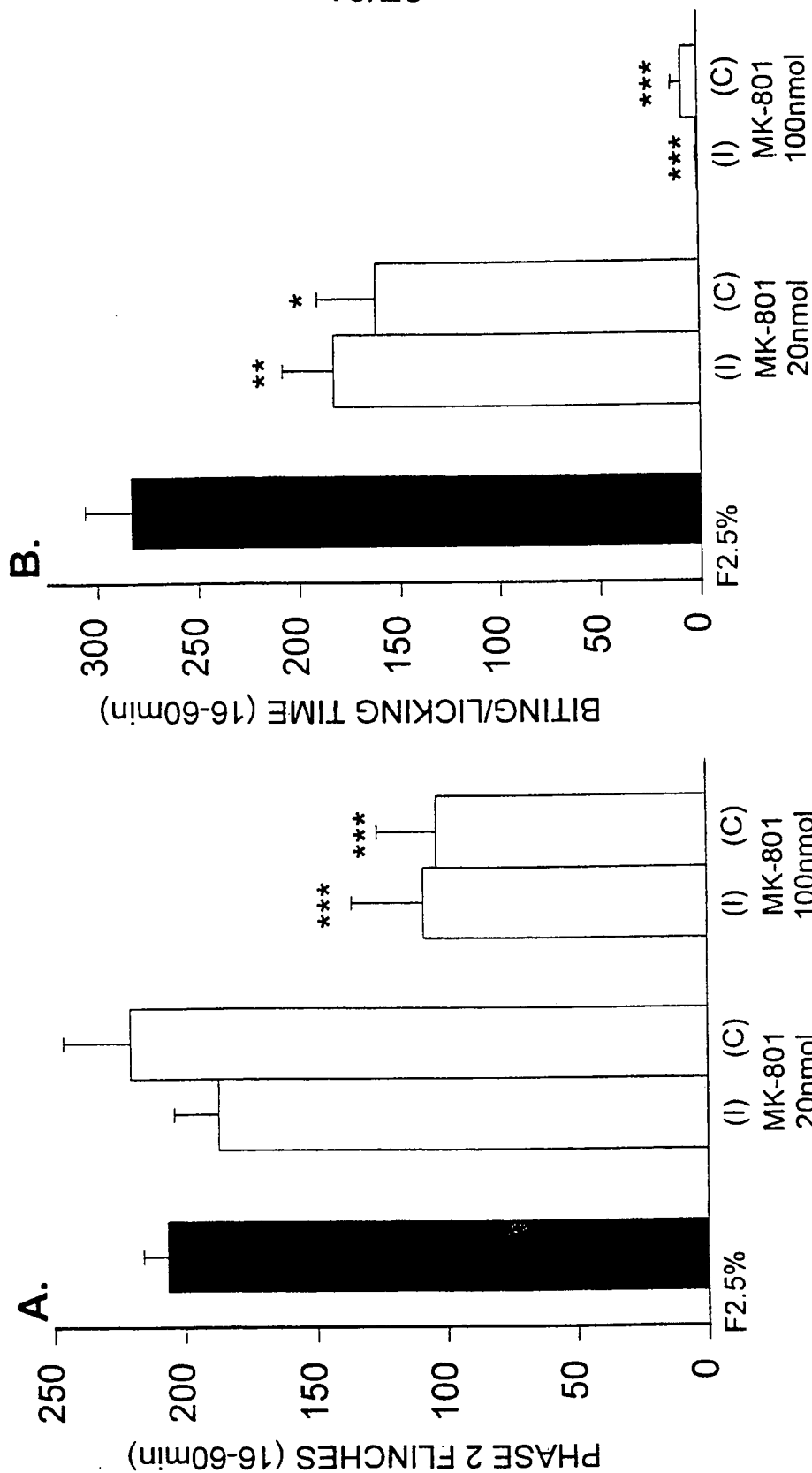
FIGURE 14

FIGURE 15B

FIGURE 15A

FIGURE 16A

FIGURE 16B

FIGURE 17A

A. phase 1 (0-12)

DESIPRAMINE: ▲ IPSILATERAL
△ CONTRALATERAL
FLUOXETINE: ● IPSILATERAL
○ CONTRALATERAL

FIGURE 17B

B. phase 2 (16-60)

FIGURE 17C

C. phase 2 (16-60)
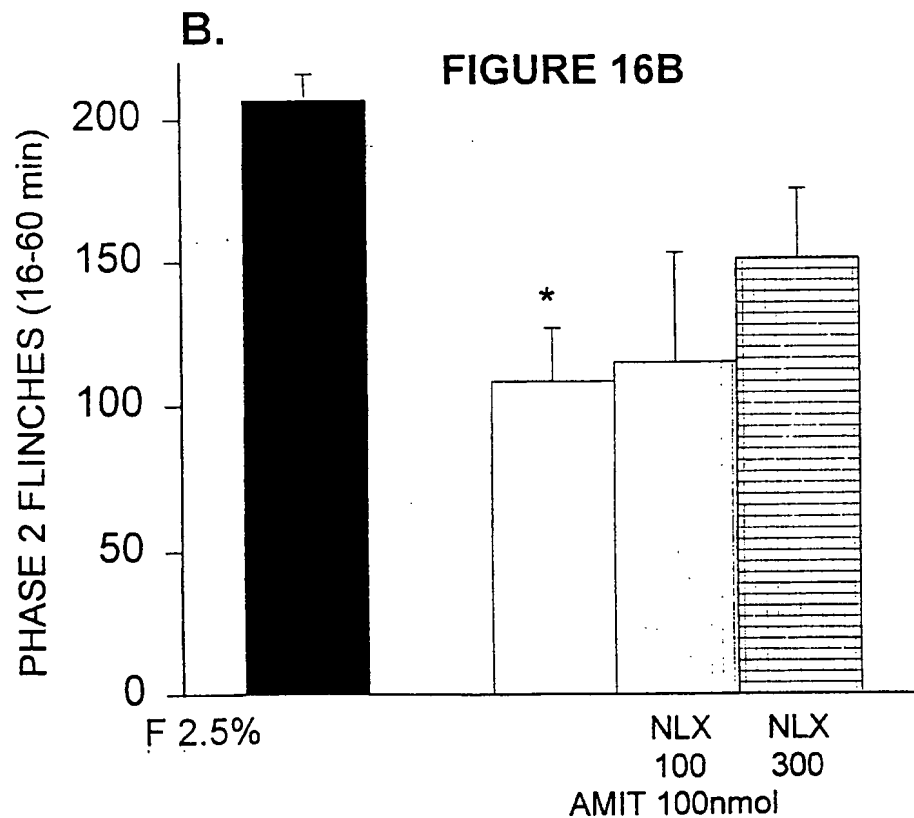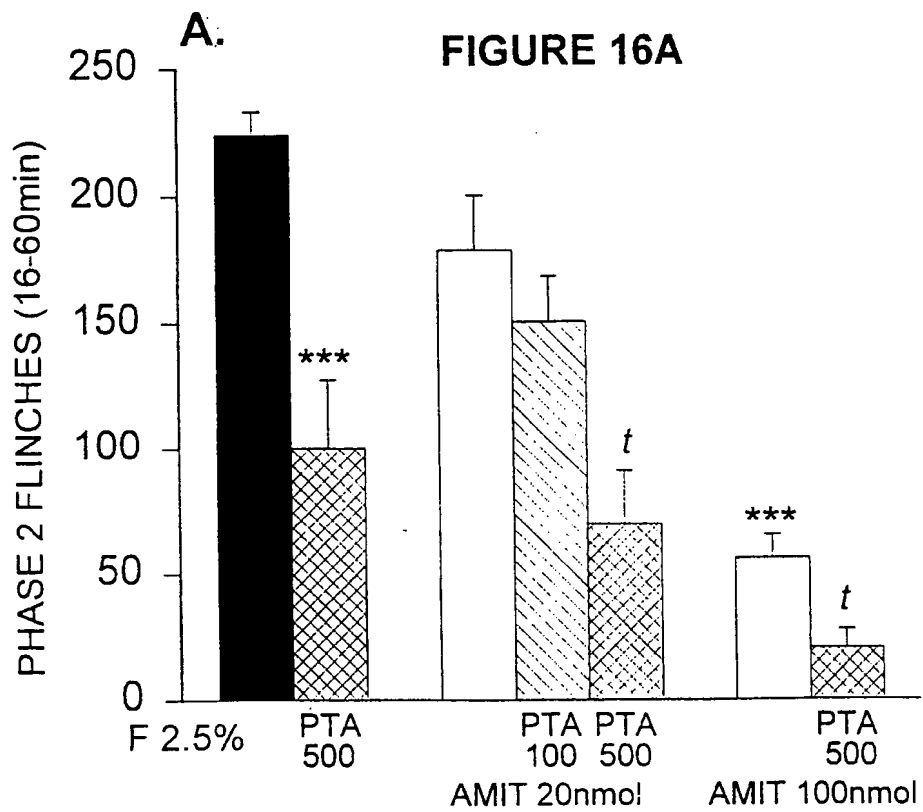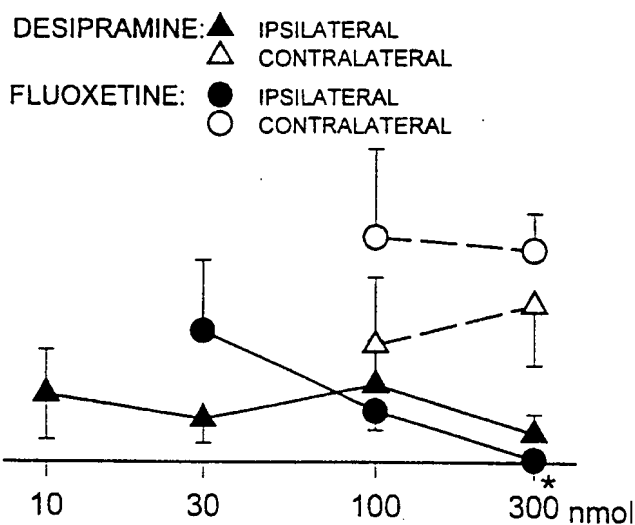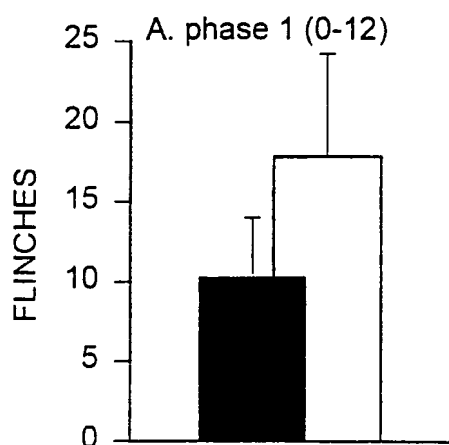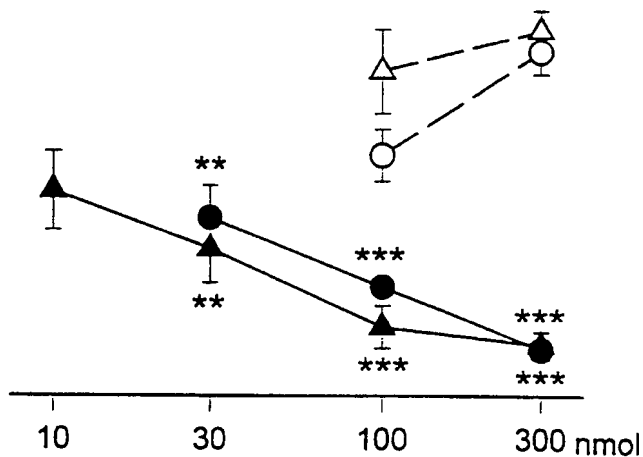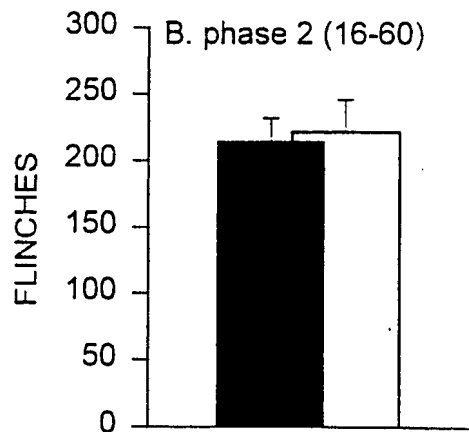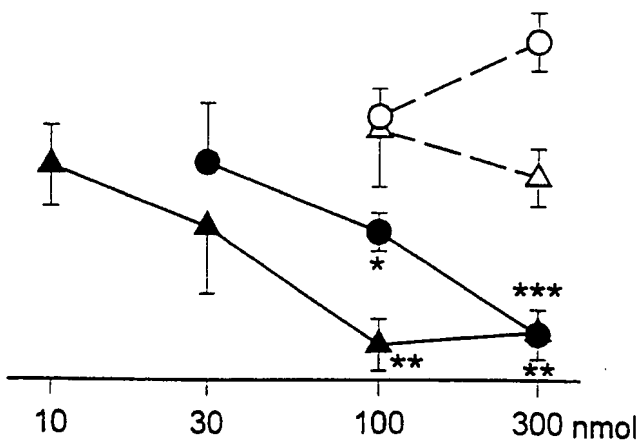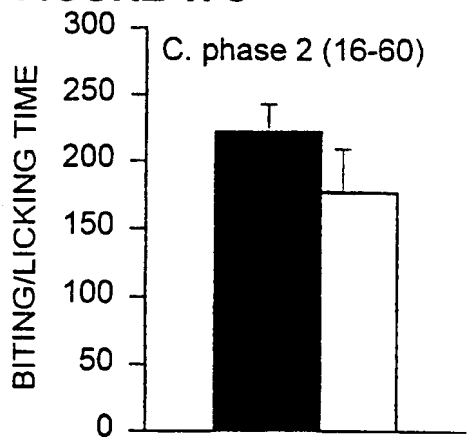
A. IPSILATERAL   **FIGURE 18A**

B. CONTRALATERAL   **FIGURE 18B**
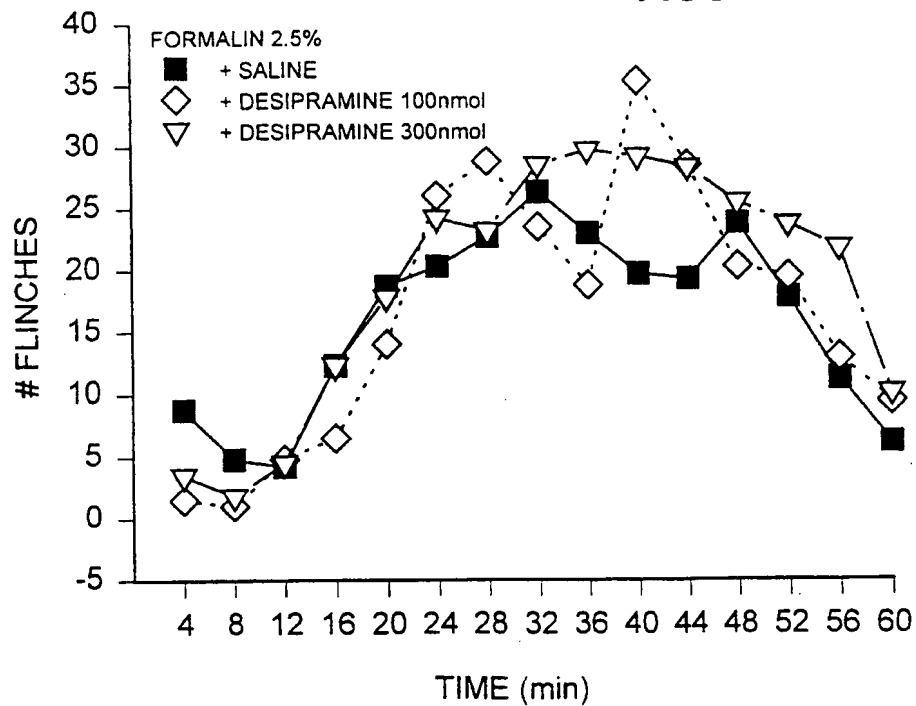
C. IPSILATERAL   **FIGURE 18C**
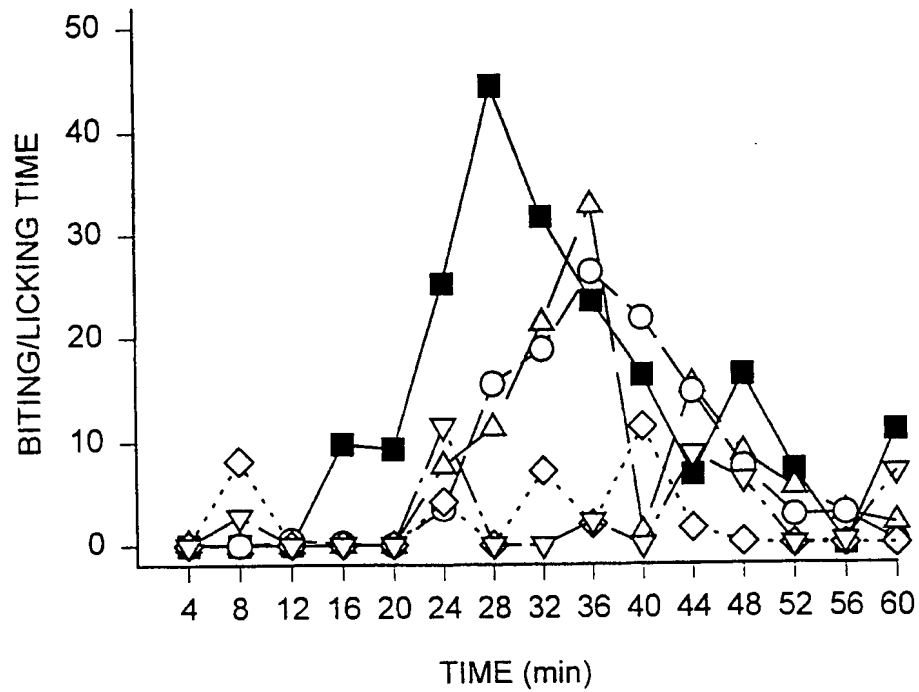
TIME (min)

D. CONTRALATERAL   **FIGURE 18D**
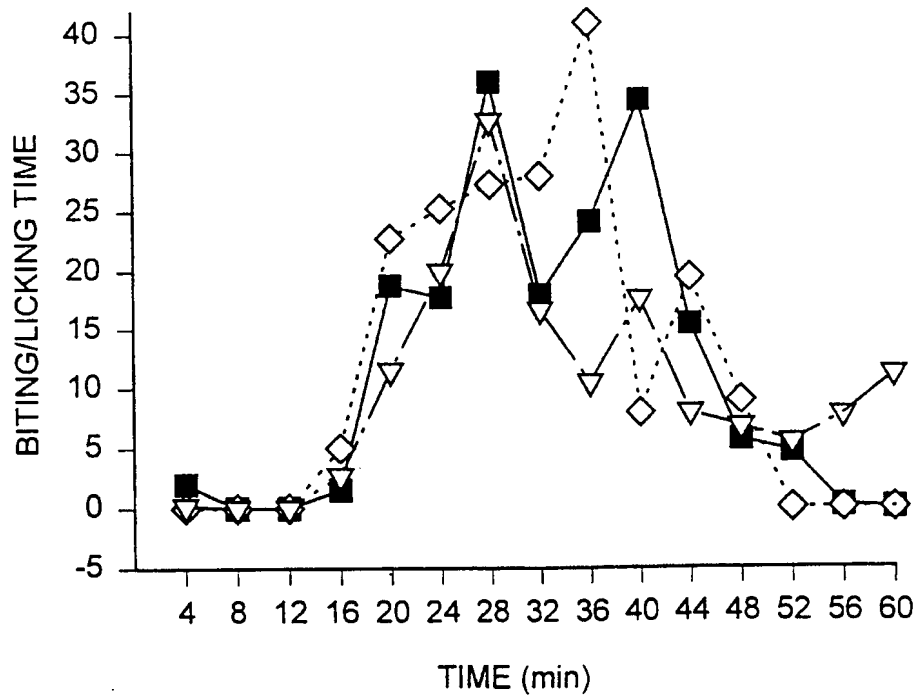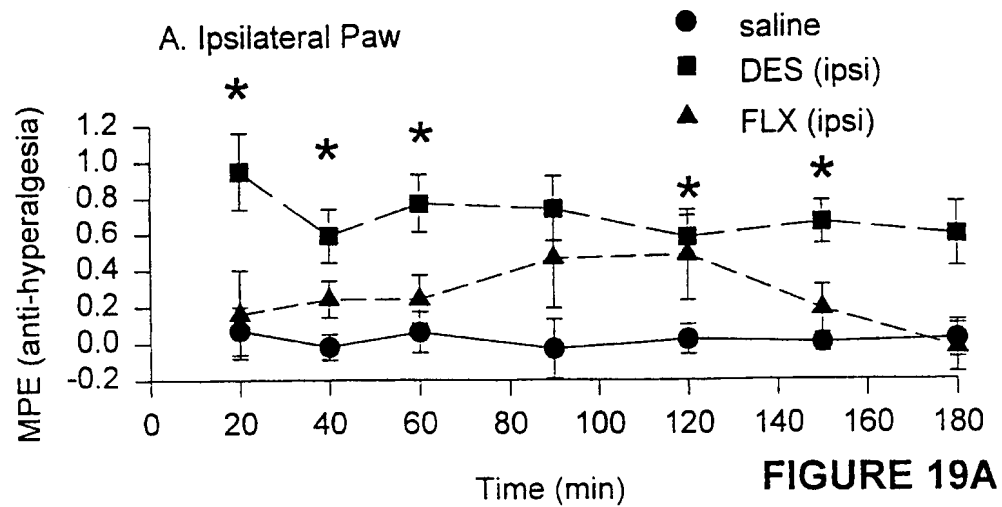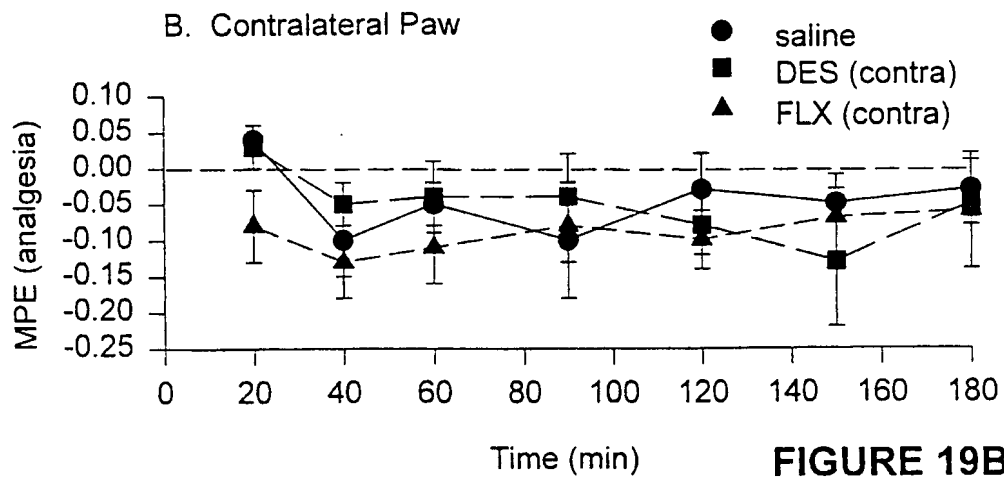
TIME (min)

A. Ipsilateral Paw

FIGURE 19A

B. Contralateral Paw

FIGURE 19B

C. Cumulative MPE (ΣMPE)

FIGURE 19C

A. Ipsilateral Paw

FIG. 20A

B. Cumulative change in MPE (ΔMPE)

FIG. 20B

A. phase 1 — FIGURE 21A

B. phase 2 — FIGURE 21B

C. phase 2 — FIGURE 21C

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B. CONTRALATERAL

FIGURE 22B

A. IPSILATERAL

FIGURE 22A

FIGURE 23B

B. FLUOXETINE

FIGURE 23A

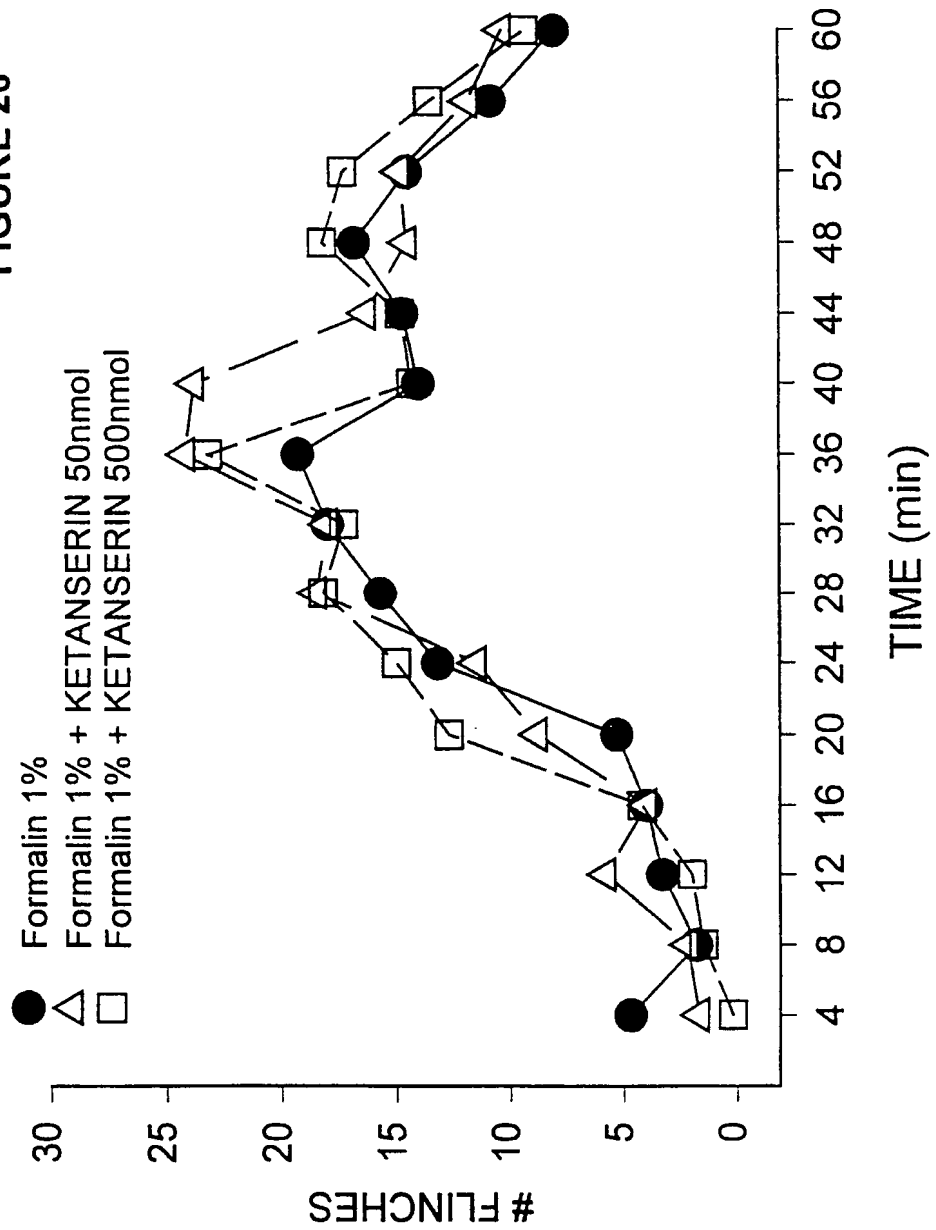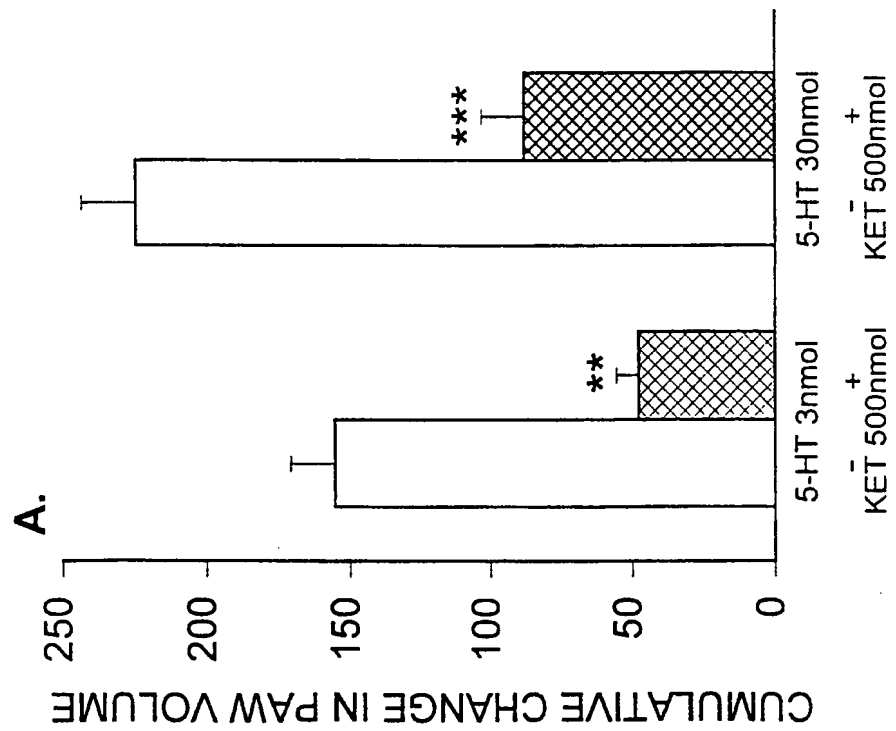FIGURE 23C

A. DESIPRAMINE

C. AMITRIPTYLINE
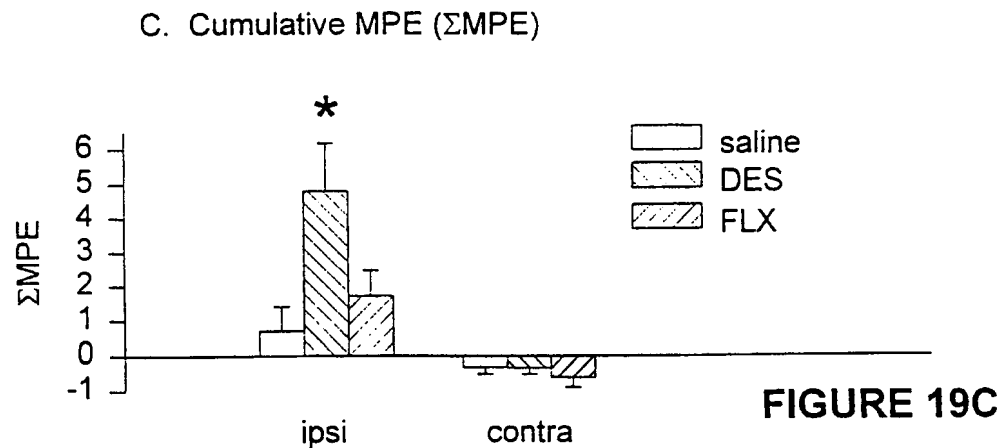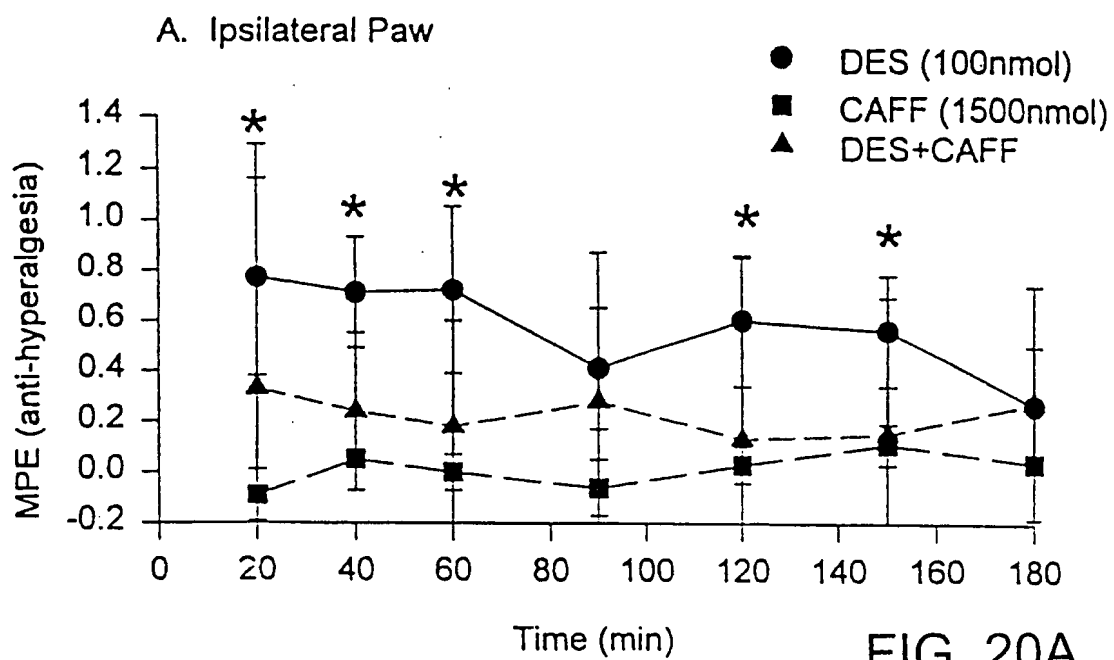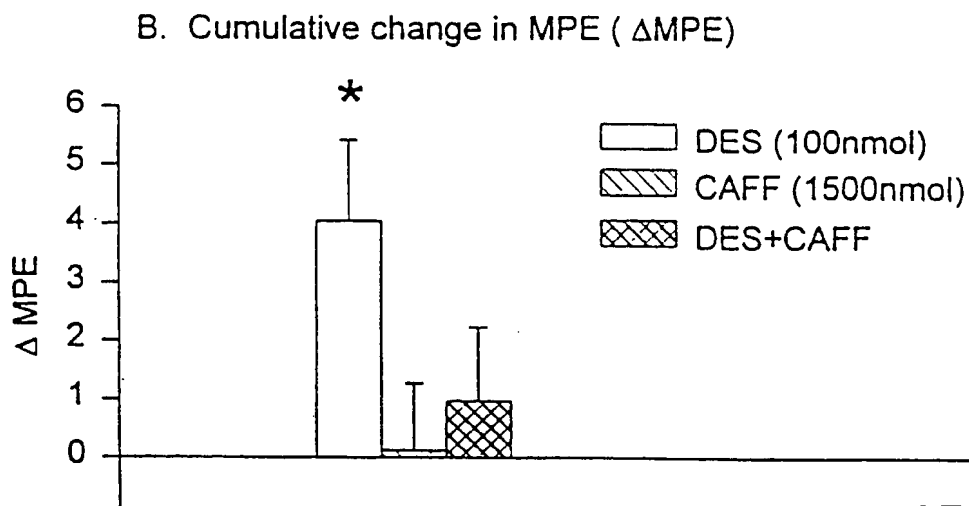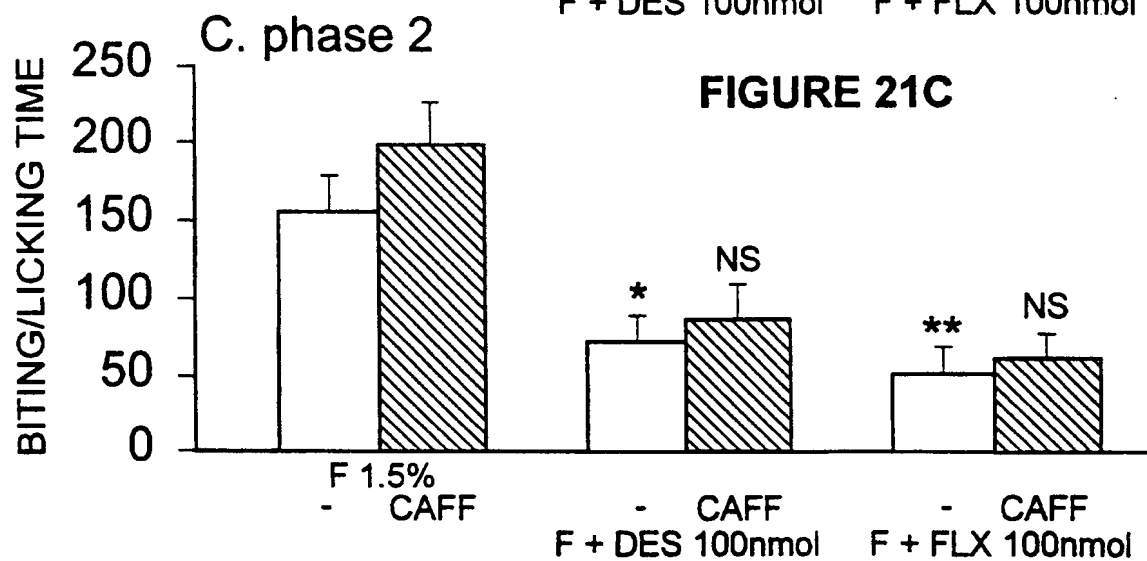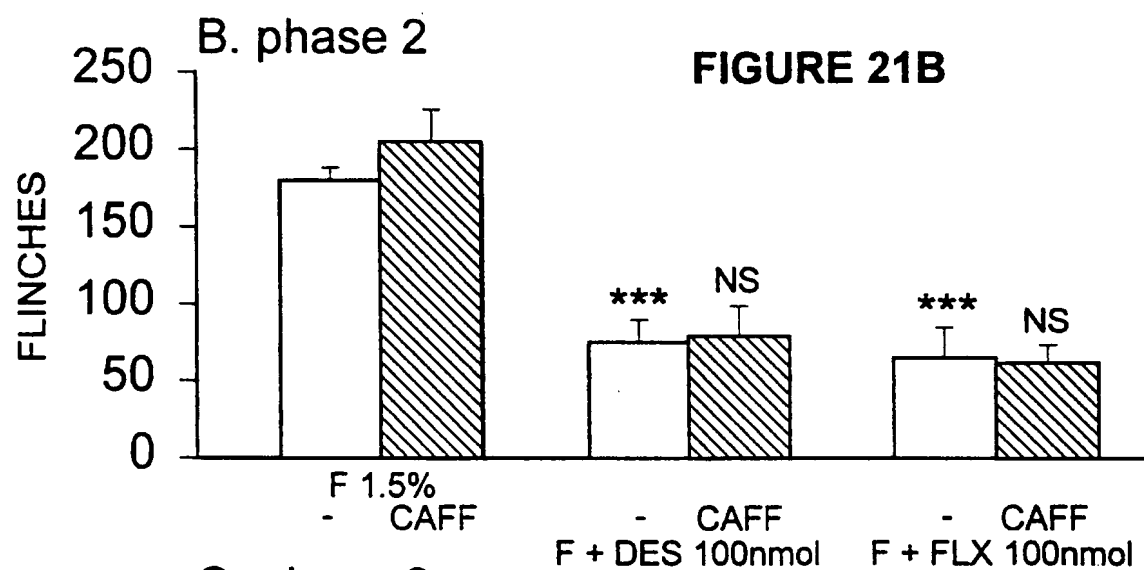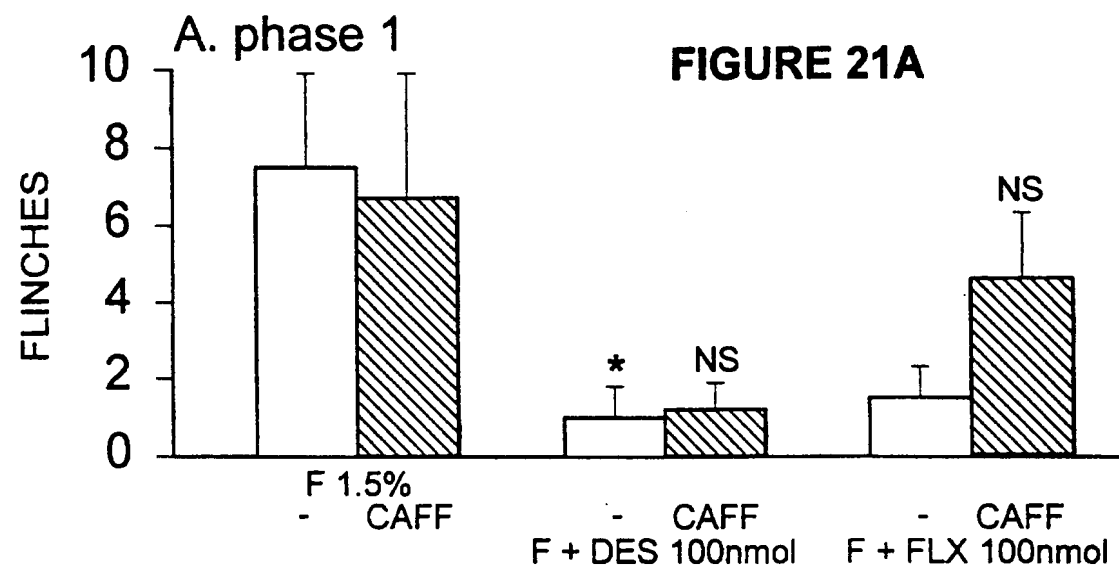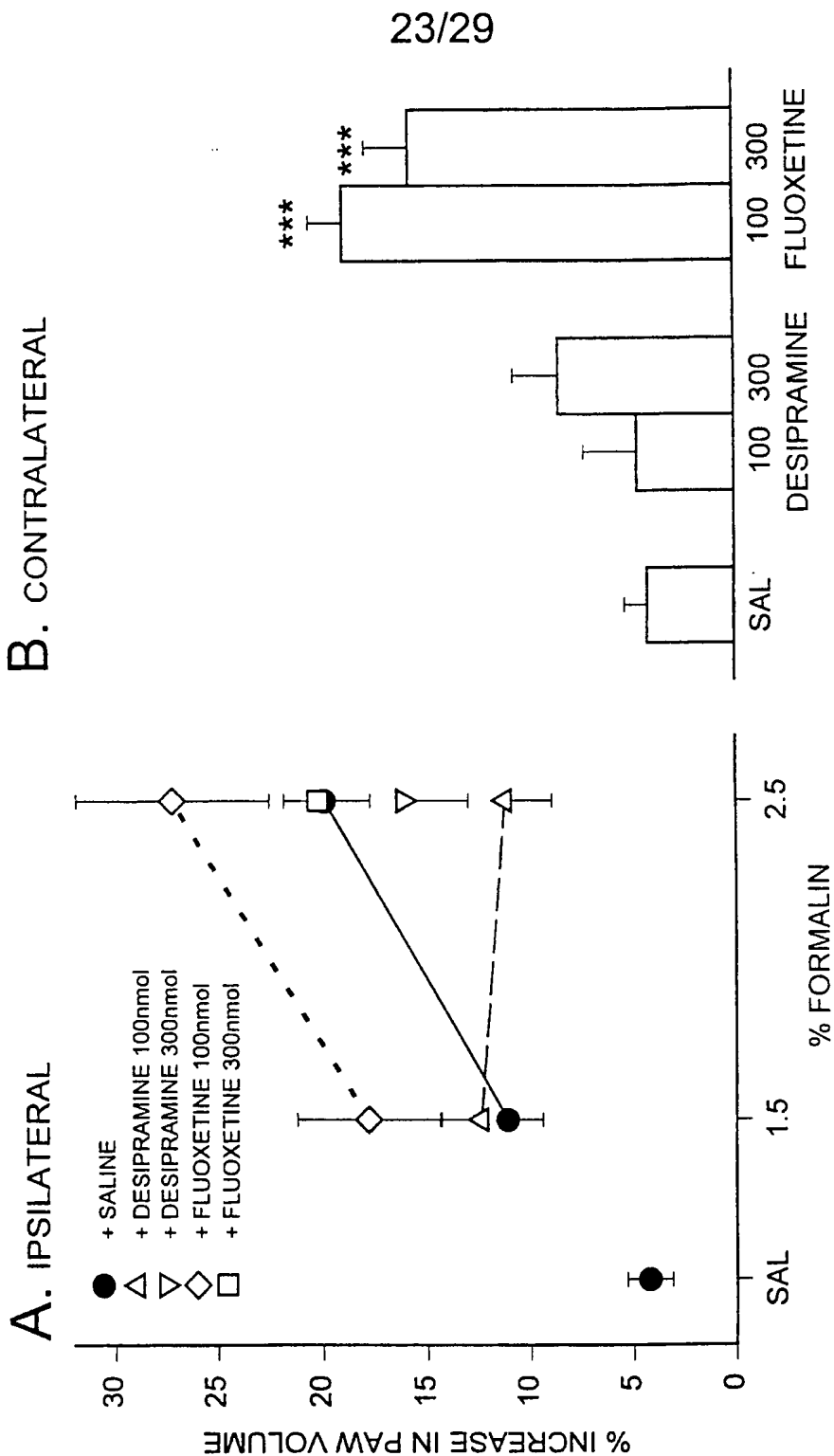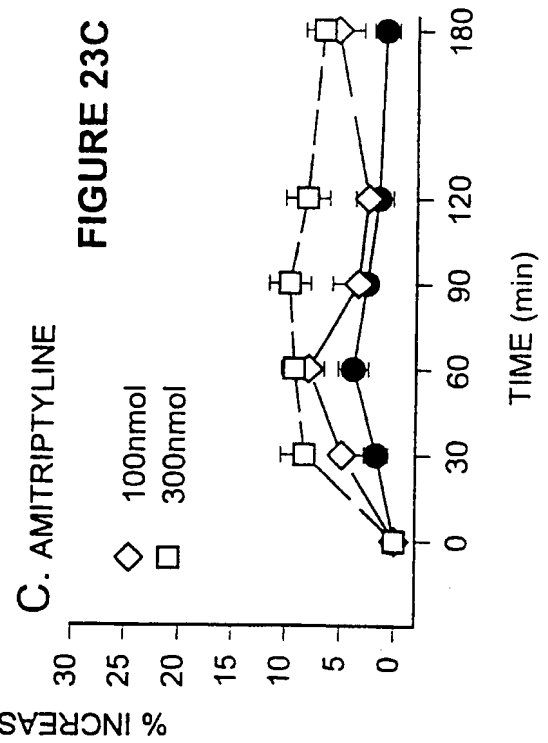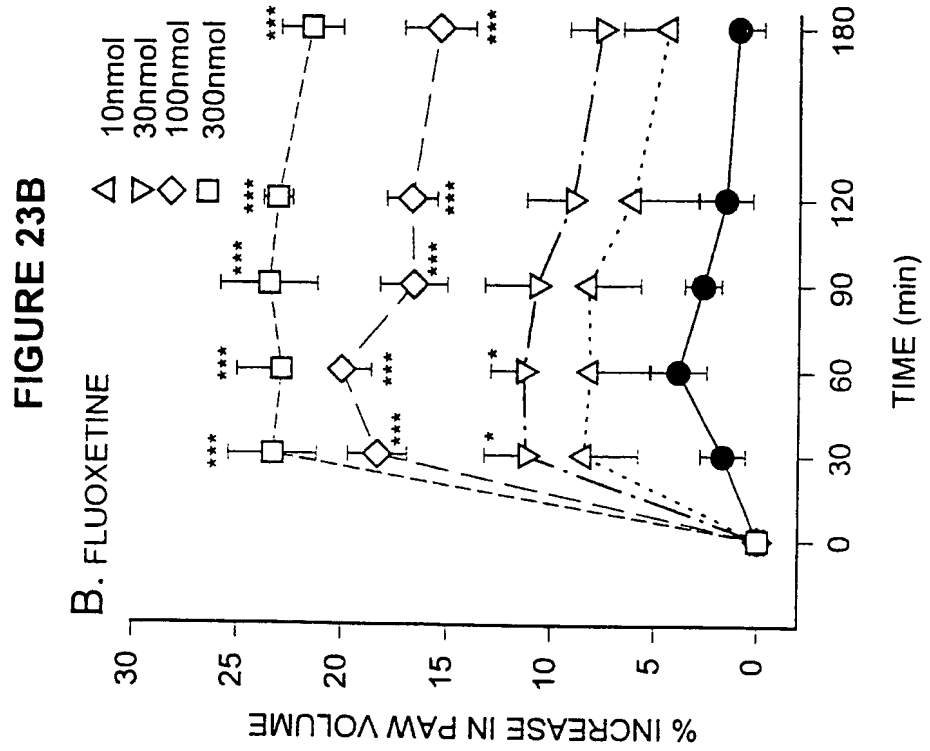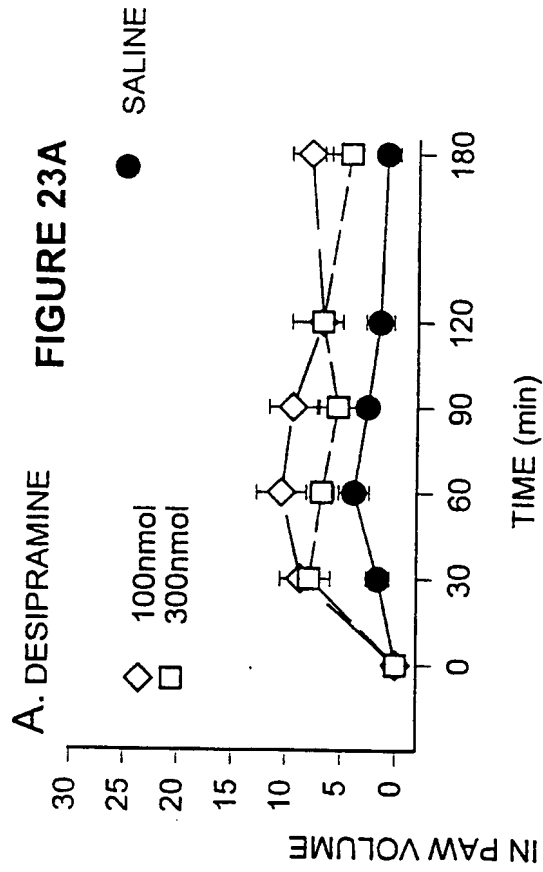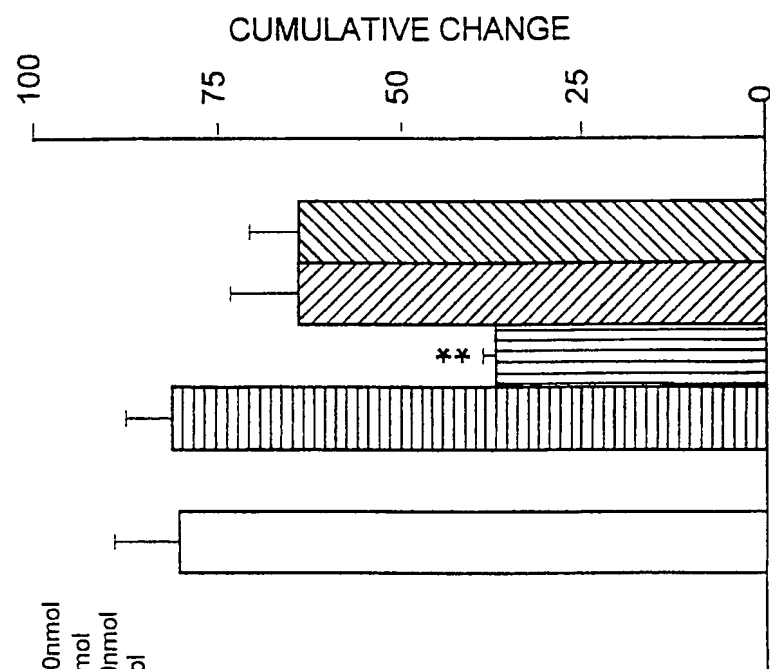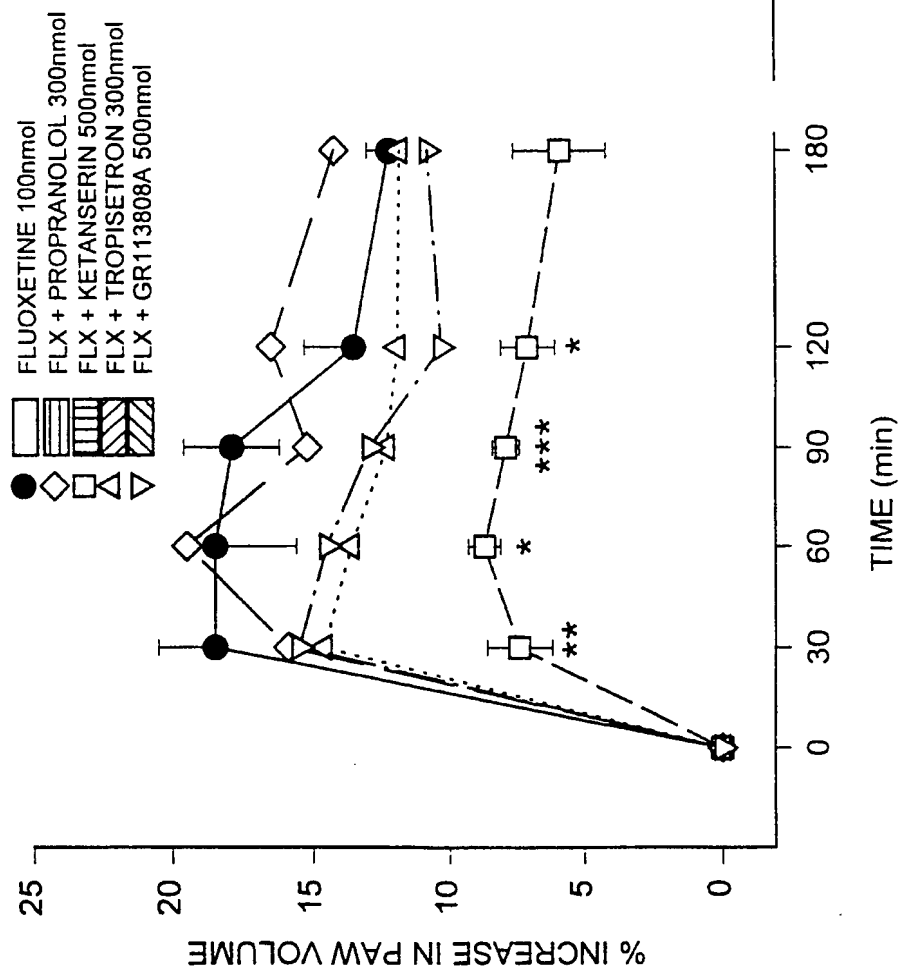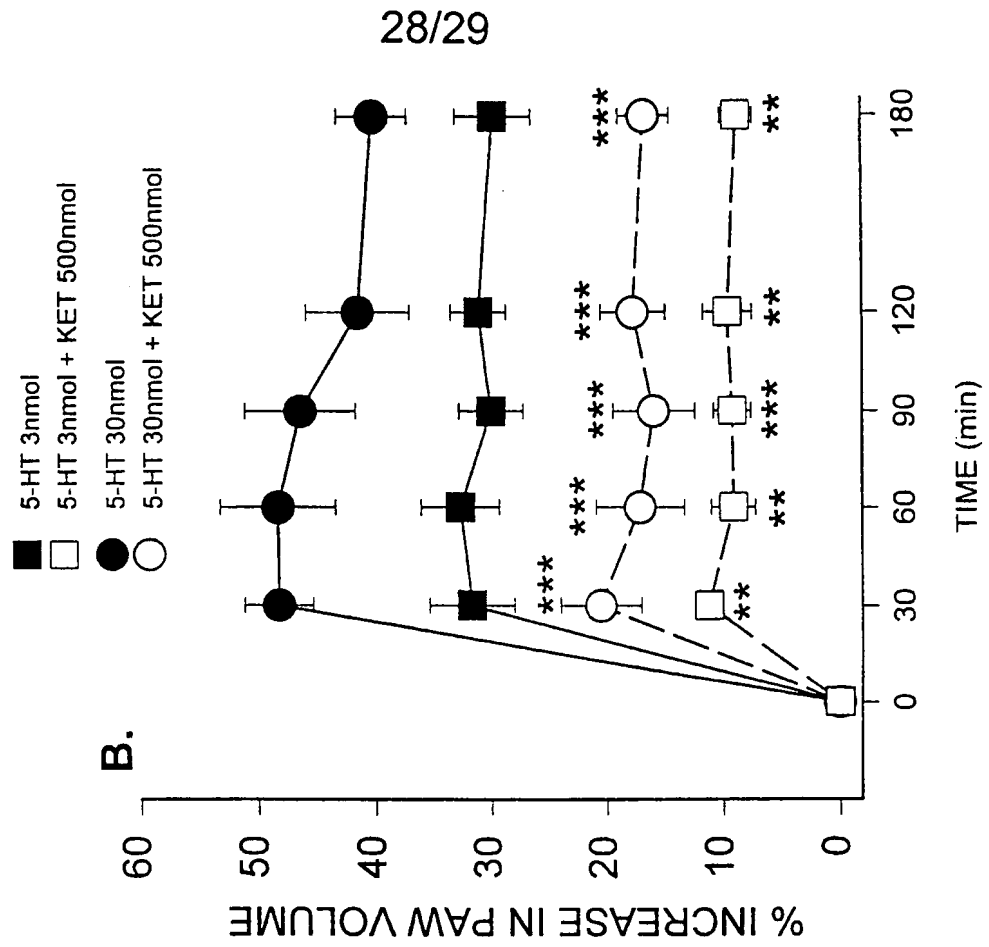
FIGURE 24B

FIGURE 24A

FIGURE 25
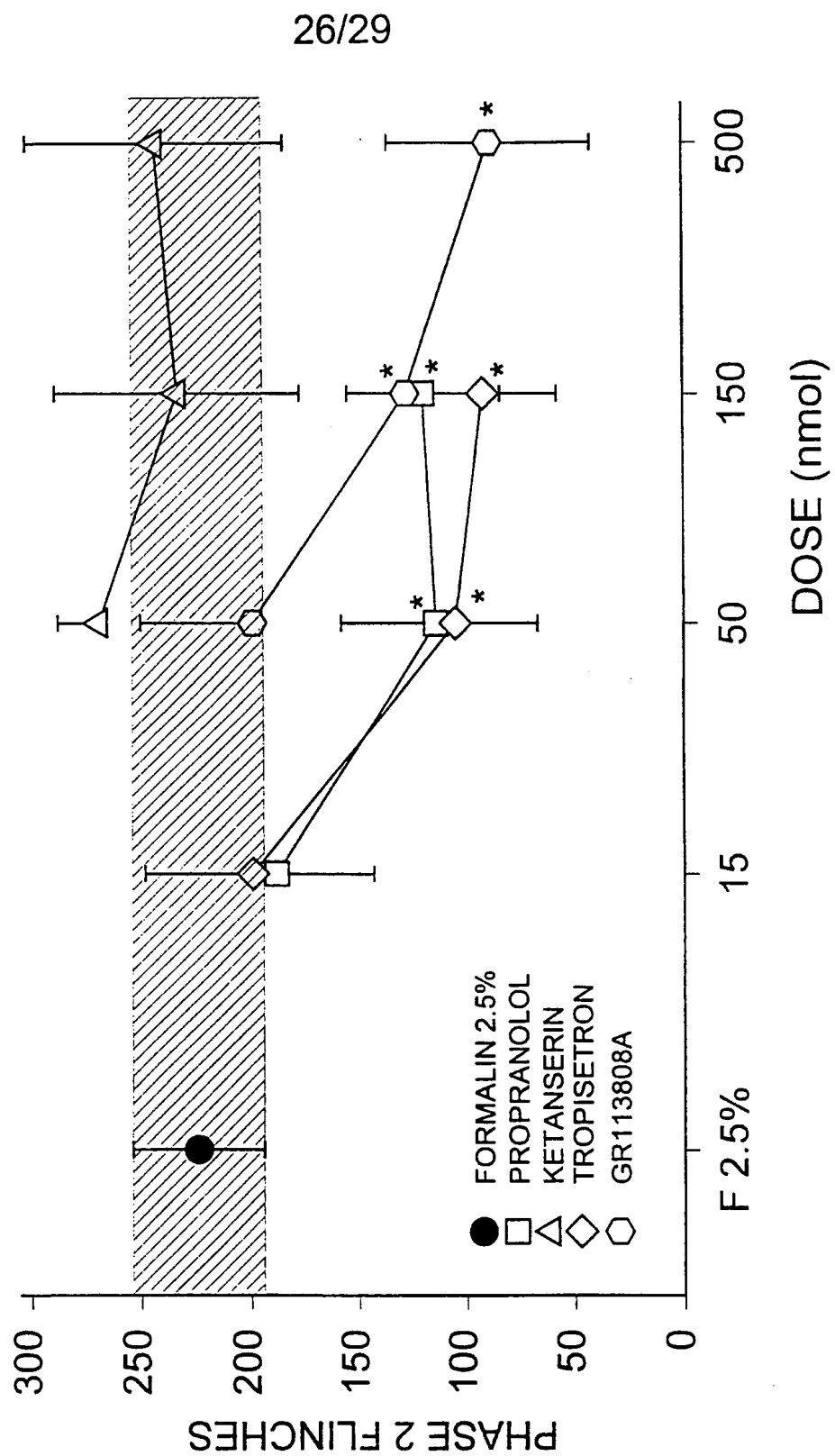
FIGURE 26

FIGURE 27B

FIGURE 27A

FIGURE 28A
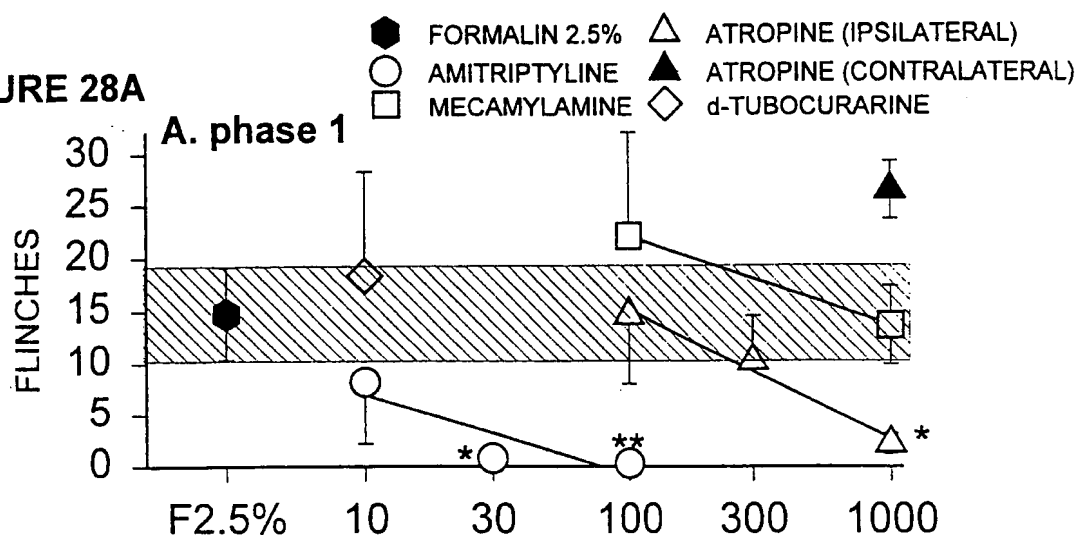
FIGURE 28B
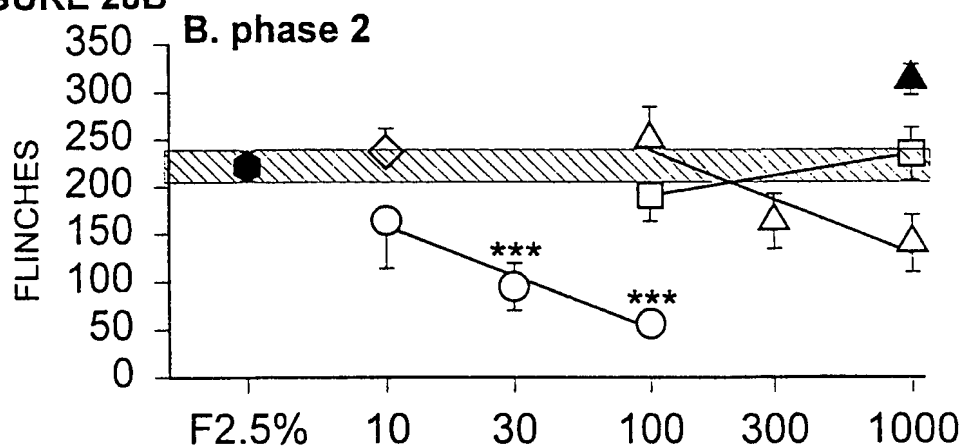
FIGURE 28C
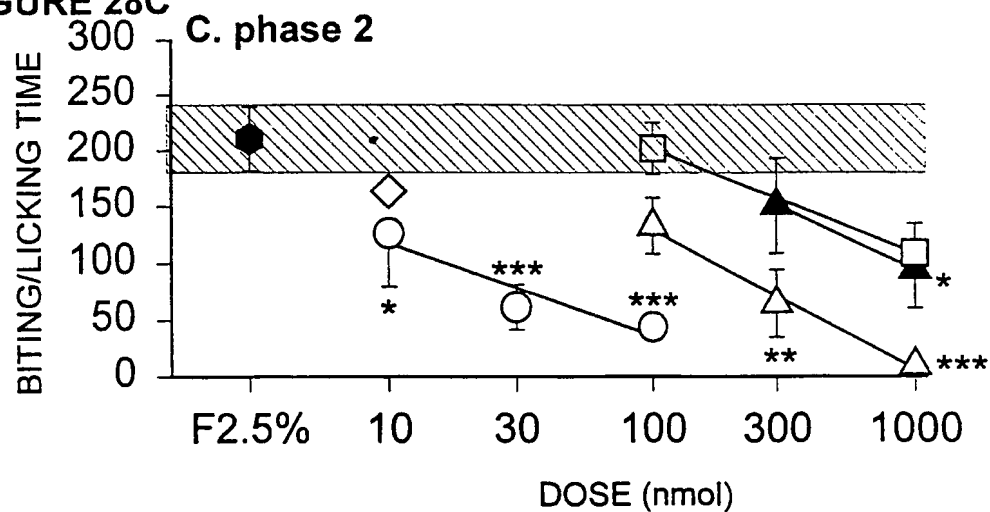
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61K31/645   A61K31/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 01157 A (THE WWK TRUST) 15 January 1998 (1998-01-15) page 1, line 4 - line 11 page 2, line 1 - page 3, line 7 page 4, line 11 - line 22 page 8, line 7 - line 19 page 11, line 23 - line 27 claims 1-5,8,9,12,13,16,19,20 --- -/--	1-53

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
13 August 1999	01/09/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Economou, D

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page 1 of 2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 10815 A (FROME, B.) 27 March 1997 (1997-03-27) claims 1,3,5,7,9,11,13,15,17,20,22 page 20, line 1 - page 31, paragraph 3 page 18, paragraph 3 - page 19, paragraph 1 page 17, last paragraph - page 18, line 3 page 16, last paragraph - page 17, paragraph 1 page 15, paragraphs 1,3 page 13, paragraph 3 page 11, last paragraph page 9 page 5, paragraph 4 page 1, line 1 - page 4, line 6 ---	1-53
X	ORTEGA-ALVARO A. ET AL.: "The effects of different monoaminergic antidepressants on the analgesia induced by spinal cord adrenal medullary transplants in the formalin test in rats" ANESTHESIA AND ANALGESIA, vol. 84, no. 4, April 1997 (1997-04), pages 816-820, XP002112201 usa page 817, left-hand column - page 820, left-hand column, line 23 page 816, right-hand column abstract ---	1-53
X	SIERRALTA, F. ET AL.: "Effect of p-Chlorophenylalanine and alpha-Methyltyrosine on the Antinociceptive Effect of Antidepressant Drugs" PHARMACOLOGY AND TOXICOLOGY, vol. 77, no. 4, October 1995 (1995-10), pages 276-280, XP002112202 denmark page 277, right-hand column, line 10 - page 279, right-hand column, paragraph 5 page 277, left-hand column, paragraphs 4,6 page 276, left-hand column, paragraph 3 - page 276, right-hand column, paragraph 3 abstract -----	1-53

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9801157	A	15-01-1998	AU	3451797 A	02-02-1998
WO 9710815	A	27-03-1997	NONE		